JCN Open Access ORIGINAL ARTICLE

pISSN 1738-6586 / eISSN 2005-5013 / J Clin Neurol 2024;20(4):402-411 / https://doi.org/10.3988/jcn.2023.0435

Unusual Voltage-Gated Sodium and Potassium Channelopathies Related to Epilepsy

Hui Jin Shin
Ara Ko
Se Hee Kim
Joon Soo Lee
Hoon-Chul Kang

Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Korea

Background and Purpose There is extensive literature on monogenic epilepsies caused by mutations in familiar channelopathy genes such as *SCN1A*. However, information on other less-common channelopathy genes is scarce. This study aimed to explore the genetic and clinical characteristics of patients diagnosed with unusual voltage-gated sodium and potassium channelopathies related to epilepsy.

Methods This observational, retrospective study analyzed pediatric patients with epilepsy who carried pathogenic variants of unusual voltage-gated sodium and potassium channelopathy genes responsible for seizure-associated phenotypes. Targeted next-generation sequencing (NGS) panel tests were performed between November 2016 and June 2022 at Severance Children's Hospital, Seoul, South Korea. Clinical characteristics and the treatment responses to different types of antiseizure medications were further analyzed according to different types of gene mutation.

Results This study included 15 patients with the following unusual voltage-gated sodium and potassium channelopathy genes: *SCN3A* (n=1), *SCN4A* (n=1), *KCNA1* (n=1), *KCNA2* (n=4), *KCNB1* (n=6), *KCNC1* (n=1), and *KCNMA1* (n=1). NGS-based genetic testing identified 13 missense mutations (87%), 1 splice-site variant (7%), and 1 copy-number variant (7%). Developmental and epileptic encephalopathy was diagnosed in nine (60%) patients. Seizure freedom was eventually achieved in eight (53%) patients, whereas seizures persisted in seven (47%) patients.

Conclusions Our findings broaden the genotypic and phenotypic spectra of less-common voltage-gated sodium and potassium channelopathies associated with epilepsy.

Keywords channelopathy; epilepsy; genetics.

INTRODUCTION

It has been reported that 738 genes are associated with epilepsy, with approximately 45% of these genes being involved in the function of ion channels, primarily sodium- and potassium-channels.[1] Voltage-gated sodium channels are involved in the initiation and conduction of action potentials in neurons, while voltage-gated potassium channels play important roles in setting the inward-negative resting membrane potential, modulating action-potential firing, and regulating neurotransmitter release.[2,3] Despite progress in the field of channelopathies related to epilepsy, further studies are needed to fully understand the pathomechanisms of gene mutations, clinical characteristics of patients, and effective treatment methods.

This study aimed to determine 1) the genetic and clinical aspects of unusual voltage-gated sodium and potassium channelopathies related to epilepsy and 2) the therapeutic efficacies of different types of antiseizure medications (ASMs) according to different types of

Received October 24, 2023
Revised January 3, 2024
Accepted January 23, 2024

Correspondence
Hoon-Chul Kang, MD, PhD
Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel +82-2-2228-2075
Fax +82-2-393-9118
E-mail hipo0207@yuhs.ac

gene mutation.

METHODS

Study design and patient selection

This observational retrospective study analyzed pediatric patients diagnosed with epilepsy who underwent targeted next-generation sequencing (NGS) between November 2016 and June 2022 at Severance Children's Hospital, Seoul, South Korea. The inclusion criteria were as follows: 1) epilepsy diagnosis, 2) seizure onset ≤18 years, 3) carrier of pathogenic or likely pathogenic variants in unusual voltage-gated sodium- and potassium-ion-channel genes responsible for epilepsy or seizure-associated phenotypes, and 4) clinical follow-up of >1 year. Patients with metabolic, infectious, immunological, or other etiologies of epilepsy (e.g., trauma) were excluded. This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine (4-2023-1084). The requirement to obtain informed consents was waived due to the retrospective design of the study.

Gene testing

NGS-based genetic testing was performed in all patients. A customized epilepsy gene panel comprising 172 candidate genes was utilized when patients were diagnosed with specific epilepsy syndromes that might be associated with developmental and epileptic encephalopathy (DEE). Whole exome sequencing (WES) was used when patients presented with seizures and developmental delay, without diagnoses of specific epilepsy syndromes; WES comprised of the xGen Inherited Diseases Panel (Integrated DNA Technologies, Coralville, IA, USA) comprising 4,503 genes.

Genomic DNA extracted from the peripheral blood of patients using the QIAamp Blood DNA Mini Kit (Qiagen, Hilden, Germany) was analyzed using the MiSeq sequencer (Illumina, San Diego, CA, USA) and quantified with the Qubit HS dsDNA kit (Invitrogen, Carlsbad, CA, USA). Other customized gene panel sequencing libraries were prepared using a DxSeq customized gene panel for the Illumina Platform Kit (Dxome, Seoul, South Korea) according to the manufacturer's recommendations, followed by 300 cycles of paired-end sequencing on the NovaSeq 6000 System (Illumina). The GRCh37 (hg19) was used to identify DNA/RNA sequence variants and protein sequences. We have previously described the methodology used to detect variants.[4-6] Additionally, the online SpliceAI server (https://spliceailookup.broadinstitute.org/) was used to predict the splicing effects of variants. SpliceAI scores range from 0 to 1, with higher scores indicating a higher probability of splicing being affected.

Analyses and variant annotations were performed using the following online databases: the Human Gene Mutation Database, Online Mendelian Inheritance in Man, ClinVar, dbSNP, 1000 Genomes, Exome Aggregation Consortium, Exome Sequencing Project, Korean Reference Genome Database, and Leiden Open Variation Database. Variants were classified according to standards and guidelines for interpreting sequence variants established by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[7] Variants were described using nomenclature of the Human Genome Variation Society. If further clinical correlation was required, a consensus discussion of genotype–phenotype correlations was performed among geneticists, pediatric neurologists, and laboratory diagnosticians, along with additional family studies or confirmatory assays.

Where necessary, parental studies were conducted using either 1) an NGS-based trio test using the NextSeq 550Dx System (Illumina) combined with analysis using the custom bioinformatics pipeline or 2) a Sanger sequencing trio test using the 3730 DNA analyzer with the BigDye Terminator (version 3.1) Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). A protein topology plot was generated using Protter software (version 1, http://wlab.ethz.ch/protter).[8]

Clinical characteristics

Epilepsy syndromes, drug-resistant epilepsy (i.e., refractory), and DEE were defined and classified according to the definitions and classifications of the International League Against Epilepsy.[9-11] Effective treatment was defined as a >50% reduction in seizure frequency for at least 3 months after treatment initiation. Epilepsy outcomes were assessed according to Brodie's pattern of treatment responses in epilepsy (Table 1).[12] Patients were initially treated with ASM monotherapy, and then subsequently with adjuvant ASMs if their seizures persisted. The ASMs comprised of sodium-channel blockers (SCBs) such as phenytoin (PHT), carbam-

Table 1. Brodie's pattern of treatment responses in epilepsy

Seizure outcome	Description
Early seizure freedom	Seizure freedom immediately or ≤6 months after starting treatment
Delayed seizure freedom	Seizure freedom after >6 months of treatment but remained seizure-free through follow-up
Fluctuating	Periods of seizure freedom lasting >1 year interspersed with relapses
Refractory	No seizure freedom during any complete year

azepine (CBZ), oxcarbazepine (OXC), rufinamide (RUF), and lacosamide (LCS); zonisamide (ZNS), topiramate (TPM), and pregabalin (PGB), which may act as calcium-channel blockers (CCBs); phenobarbital (PB) and clobazam (CLB), which are predominant GABAergic ASMs; and levetiracetam (LEV) and valproic acid (VPA), which are broad-spectrum ASMs. Refractory epilepsy patients were considered for treatment with cannabidiol (CBD), high-dose steroids, the ketogenic diet, or even corpus callosotomy if applicable.

Statistical analyses

Descriptive statistics were used for the clinical and genetic characteristics, with range, mean, and standard-error-of-the-mean values reported as accordingly, using Office LTSC Professional Plus 2021 by Microsoft.

RESULTS

Patients

Overall 495 (23.4%) of the 2,114 initially enrolled patients were found to have likely pathogenic or pathogenic variants. Mutations in genes related to voltage-gated channelopathies were present in 162 (32.7%) of these patients, while 15 (3.0%) had the following unusual voltage-gated sodium- and potassium-channel genes: *SCN3A* (*n*=1), *SCN4A* (*n*=1), *KCNA1* (*n*=1), *KCNA2* (*n*=4), *KCNB1* (*n*=6), *KCNC1* (*n*=1), and *KCNMA1* (*n*=1). Of the 15 variants, one was novel (*SCN3A*); the rest were reported previously (five by our institution and the rest by other studies). We describe the clinical characteristics and also compare their similarities and differences with nine previously identified pathogenic variants in Table 2.

Sodium channelopathies

SCN3A

We report a novel pathogenic splice-site variant of *SCN3A* that was previously reported as a likely benign variant in ClinVar. This variant in intron 18 of *SCN3A* is considered to be likely pathogenic. Its SpliceAI score is 0.99, and it is not present in population databases. In strict adherence to American College of Medical Genetics and Genomics guidelines, this intronic variant may be observed as a variant of unknown significance. Unfortunately, the patient's family did not agree to further genetic testing such as parental genetic studies including NGS-based or Sanger-sequencing trio tests, which might have helped to confirm the pathogenicity. However, the patient displayed evident clinical manifestations of DEE, which is consistent with previous reports;[13] this allows our intronic variant to be characterized as likely pathogenic in the clinical setting.

Our patient developed spasms during infancy that presented electroclinically as infantile epileptic spasm syndrome that evolved into Lennox-Gastaut syndrome (LGS). Magnetic resonance imaging revealed no abnormalities. The patient's epilepsy was refractory. Patient management that included multiple ASMs, high-dose steroids, the ketogenic diet, and even corpus callosotomy was ineffective in reducing the seizures. Ultimately, ZNS and RUF were effective at reducing the seizure frequency by >50%, with seizure freedom being achieved after adding CBD.

SCN4A

We present the first pathogenic variant of *SCN4A* associated with epilepsy. This was a missense pathogenic variant and located in membrane segment S4 of domain II. This pathogenic variant was previously reported to be the only variant associated with normokalemic periodic paralysis.[14] Our patient was diagnosed with early infantile developmental and epileptic encephalopathy (EIDEE), with myoclonic seizure onset at age 2 weeks and the subsequent development of absence seizures. Initial electroencephalogram (EEG) showed nearly continuous multifocal epileptiform discharges. The patient eventually showed delayed seizure freedom after administering PB, TPM, and PGB. SCBs such as OXC, PHT, and LCS were ineffective, as was the ketogenic diet.

Potassium channelopathies

KCNA1

We identified a patient with a pathogenic missense variant in *KCNA1* located in the pore helix between segments S5 and S6. This variant has been reported previously but without a specific clinical description.[15] This patient developed seizures during infancy, subsequently experienced multiple seizure types, and was ultimately diagnosed with LGS. The patient developed spastic dystonia. Despite their seizures being intractable, significant seizure reductions were achieved with RUF coadministered with VPA and the ketogenic diet.

KCNA2

We identified four patients with missense mutations in *KCNA2*, all of which were located between segments S3 and S6 (Fig. 1). Two siblings shared the same variant (c.1250A>G), which has been reported previously.[16] They exhibited normal development but experienced generalized tonic and absence seizures, with EEG showing generalized epileptiform discharges.

The other two patients with *KCNA2* missense variants, reported previously by our institution, showed generalized tonic or tonic–clonic seizures.[17] Their EEG findings showed focal epileptiform discharges. One of the patients showed

Table 2. Clinical and genetic characteristics of sodium and potassium channelopathies

Patient	Sex/ age	Gene	Nucleotide/ protein change	Channel segments/ domain	Seizure onset age	Seizure semiology/ syndrome	Development/ others	EEG	MRI	Outcome	Effective to SCB?	Effective ASM	Reference
P1	M/6 y	*SCN3A* (NM_006922.3)	c.3393+2T>G		1 y 1 mo	Spasm, GT/IESS, LGS	N>DD	SDBG, GSSW, multifocal ED	N	Delayed seizure freedom	Yes	ZNS, RUF, CBD	This study
P2	M/9 y	*SCN4A* (NM_000334.4)	c.2023C>T (p.Arg675Trp)	S4 of domain II	14 d	Myoclonic, absence/EIDEE	DD	SDBG, multifocal ED	N	Delayed seizure freedom	No	PB, TPM, PGB	Vicart et al.,[14] 2004
P3	M/19 y	*KCNA1* (NM_000217.2)	c.1112C>T (p.Thr371Ile)	Pore helix between S5 and S6	10 mo	GT, myoclonic, absence/LGS	N>DD, spastic dystonia	SDBG, GSSW, multifocal ED	N	Refractory	Yes	RUF, VPA, KD	Ko et al.,[15] 2018
P4	M/2 y	*KCNA2* (NM_004974.3)	c.1250A>G (p.Tyr417Cys)	S6	6 mo	GT	N	Generalized ED	N	Early seizure freedom	Yes	OXC	Döring et al.,[16] 2021
P5	F/11 y	*KCNA2* (NM_004974.3)	c.1250A>G (p.Tyr417Cys)	S6	10 y	Absence, GT	N	Generalized ED	N	Early seizure freedom	Yes	OXC	Döring et al.,[16] 2021
P6	F/13 y	*KCNA2* (NM_004974.3)	c.1130A>G (p.Tyr377Cys)	Transmembrane domain near pore helix between S5 and S6	7 y 7 mo	GT	N	SDBG, focal ED	Arachnoid cyst	Delayed seizure freedom	Yes	OXC	Kim et al.,[17] 2022
P7	F/17 y	*KCNA2* (NM_004974.3)	c.785C>T (p.Ala262Val)	S3	2 y	GTC	N>DD, ataxic gait	SDBG, focal ED	N	Delayed seizure freedom	Yes	OXC	Kim et al.,[17] 2022
P8	M/3 y	*KCNB1* (NM_004975.2)	c.1106G>T (p.Trp369Leu)	Pore helix between S5 and S6	8 mo	GT/IESS, LGS	DD	SDBG, GSSW, GPFA, multifocal ED	Brain atrophy	Refractory	No	VPA	Kim et al.,[17] 2022
P9	F/5 y	*KCNB1* (NM_004975.3)	c.916C>T (p.Arg306Cys)	S4	1 y	Myoclonic/DEE	N>DD, ataxic gait	SDBG, multifocal ED	N	Refractory	No	CLB, VPA, KD	Saitsu et al.,[18] 2015
P10	M/18 y	*KCNB1* (NM_004975.2)	c.916C>T (p.Arg306Cys)	S4	9 y	Myoclonic, GT/DEE	N>DD, ataxic gait	SDBG, multifocal ED	N	Refractory	No	CLB, VPA	Saitsu et al.,[18] 2015
P11	M/10 y	*KCNB1* (NM_004975.3)	c.934C>T (p.Arg312Cys)	S4	8 y	GTC	DD	SDBG, focal ED	N	Early seizure freedom	No	VPA	Bar et al.,[19] 2020
P12	M/10 y	*KCNB1* (NM_004975.2)	c.934C>T (p.Arg312Cys)	S4	4 y 2 mo	GT/LGS	DD, hypotonia	SDBG, GSSW, multifocal ED	N	Refractory	No	VPA	Bar et al.,[19] 2020
P13	M/14 y	*KCNB1* (NM_004975.3)	c.1237G>A (p.Val413Ile)	S6	2 y 2 mo	GTC/LGS	DD	SDBG, GPFA, multifocal ED	N	Fluctuating	No	CLB, KD	Kim et al.,[17] 2022
P14	M/2 y	*KCNC1* (NM_001112741.1)	c.1262C>T (p.Ala421Val)	S6	6 mo	Myoclonic/LGS	N>DD	SDBG, focal ED	N	Refractory	No	VPA, LEV	Cameron et al.,[21] 2019
P15	F/5 y	*KCNMA1* (NM_002247.3)	c.1807A>G (p.Thr603Ala)	Cytoplasmic, after S6	9 mo	FIAS, myoclonic	N>DD, ataxic gait	SDBG, multifocal ED	N	Delayed seizure freedom	No	CLB, TPM	ClinVar (no publication)

ASM, antiseizure medication; CBD, cannabidiol; CLB, clobazam; d, days; DD, delayed development; DEE, developmental and epileptic encephalopathy; ED, epileptiform discharges; EEG, electroencephalogram; EIDEE, early infantile developmental and epileptic encephalopathy; F, female; FIAS, focal impaired-awareness seizure; GPFA, generalized paroxysmal fast activity; GSSW, generalized slow sharp wave; GT, generalized tonic seizure; GTC, generalized tonic–clonic seizure; IESS, infantile epileptic spasm syndrome; KD, ketogenic diet; LEV, levetiracetam; LGS, Lennox-Gastaut syndrome; M, male; mo, months; MRI, magnetic resonance imaging; N, normal; OXC, oxcarbazepine; PB, phenobarbital; PGB, pregabalin; RUF, rufinamide; SCB, sodium-channel blocker; SDBG, slow and disorganized background; TPM, topiramate; VPA, valproic acid; y, years; ZNS, zonisamide.

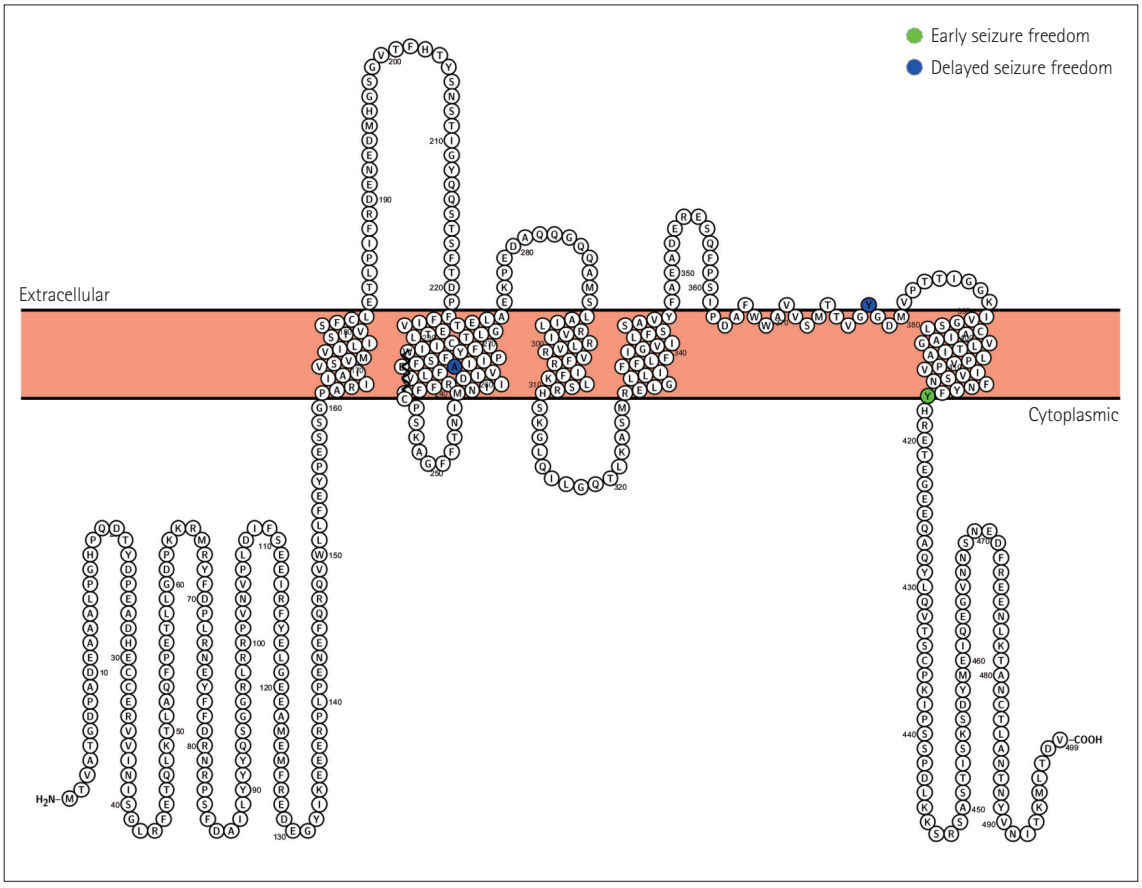

Fig. 1. Schematic diagram of *KCNA2* mutations. Pathogenic variants of *KCNA2* are shown on a protein topology plot generated using Protter software (version 1). The pathogenic variant of patient 7 (P7) was found in segment S3, with delayed seizure freedom (blue), that of P6 was located in the transmembrane domain near the pore helix between S5 and S6, with delayed seizure freedom (blue), and those of P4 and P5 were found at S6, with early seizure freedom (green).

developmental delay and an ataxic gait. It was particularly notable that all four patients had a family history of febrile seizures or epilepsy. They all responded to OXC and eventually achieved seizure freedom.

KCNB1

We detected four pathogenic missense variants in *KCNB1* in six patients. The c.916C>T and c.934C>T variants were recurrent in two patients each, both of which were reported previously.[18-20] The patients carrying the c.1106G>T and c.1237G>A variants were reported previously by our institution.[17] The c.916>T variant was located in the S4 segment, whereas the other pathogenic variants were located between segments S5 and S6 (Fig. 2).

The age at seizure onset varied. Five (83%) of the six patients were diagnosed with DEE, with three (50%) also being diagnosed with LGS. All six patients showed developmental delays. In addition, two patients had an ataxic gait, while one patient showed hypotonia. These patients experienced generalized tonic/tonic–clonic or myoclonic seizures with a broad spectrum of EEG abnormalities, ranging from focal to generalized epileptiform discharges. Five (83%) of the six patients experienced persistent seizures. None of the patients responded to SCBs such as OXC and CBZ, but five (83%) of them responded to VPA. Furthermore, three (75%) of the four patients who received CLB showed treatment responses, as did two (66%) of the three patients who were treated with the ketogenic diet.

KCNC1

We identified a patient with a missense pathogenic variant of *KCNC1* located in segment S6. This c.1262C>T variant was reported previously.[21] The patient developed myoclonic seizures at age 6 months and was eventually diagnosed with LGS. EEG revealed typical generalized slow sharp waves. Despite his epilepsy being refractory, significant seizure reduction was achieved with LEV and VPA.

KCNMA1

We identified a patient with a missense variant c.1807A>G

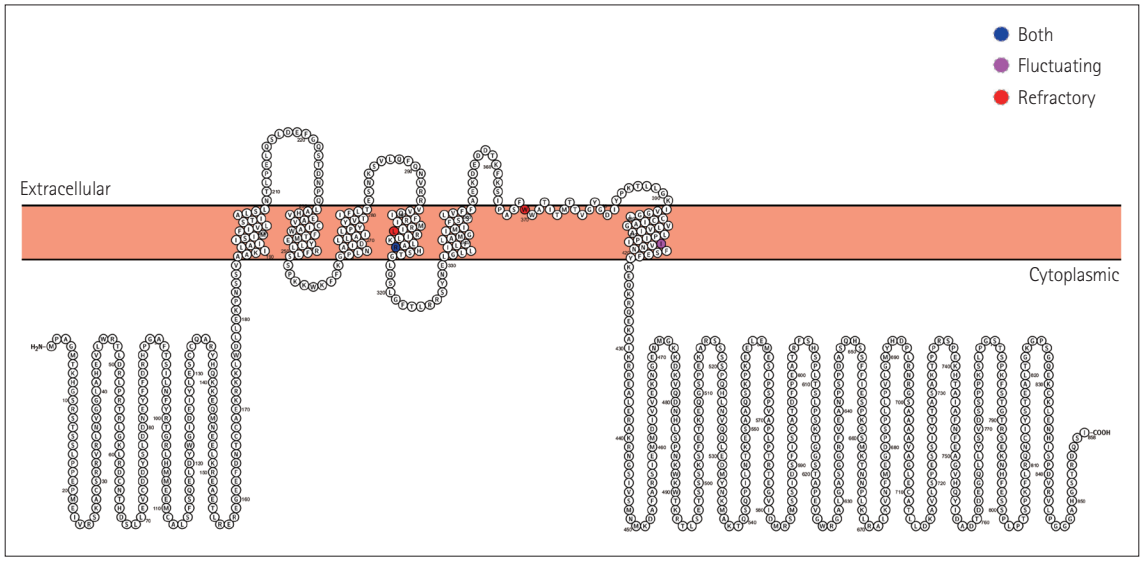

Fig. 2. Schematic diagram of *KCNB1* mutations. Pathogenic variants of *KCNB1* are shown on a protein topology plot generated using Protter software (version 1). The pathogenic variants of P9–12 were found at S4, with both fluctuating and refractory seizures (blue, red), the P8 variant was identified at the pore helix between S5 and S6, with refractory seizures (red), and the P13 variant was found at S6, with fluctuating seizures (pink). P, patient.

in *KCNMA1* located in the cytoplasmic region after segment S6. This pathogenic variant has been reported in ClinVar, but no specific studies have investigated it. Our proband developed myoclonic seizures at age 6 months and subsequently showed focal impaired awareness. EEG showed multifocal epileptiform discharges. The patient eventually achieved seizure freedom following treatment with CLB and TPM. The patient also exhibited developmental delay and an ataxic gait.

DISCUSSION

This study has demonstrated that *SCN3A*, *SCN4A*, *KCNA1*, *KCNA2*, *KCNB1*, *KCNC1*, and *KCNMA1* may be associated with epilepsy. Genetic and phenotypic heterogeneities complicate the prediction of channelopathy-associated diseases, and so further studies are required to define the clinical spectrum of these mutations. This study has revealed one new pathogenic variant in *SCN3A*, provided additional information about variants that were reported previously by our center, and compared our findings with those from other studies to add to the broad spectrum of these unusual sodium and potassium channelopathies.

Unfortunately, each variant could not be functionally validated since this is not readily possible in the clinical setting at our institution. The present study therefore focused on the clinical aspects of our patients, with the description of effective ASMs and possible hypotheses regarding their therapeutic efficacy.

SCN3A encodes the pore-forming alpha subunit of the Nav1.3 channel. This channel plays an important role in the organization, migration, and proliferation of neurons during embryonic development. Initial studies of *SCN3A* variants included missense variants associated with focal epilepsy: both gain of function (GoF) and loss of function (LoF) variants.[22,23] *SCN3A* missense variants and deletions were recently found to be associated with epileptic encephalopathy and refractory DEE in infancy, often combined with polymicrogyria and various degrees of developmental delay.[13,24,25]

To our knowledge this is the first report of a pathogenic splice-site variant in an intron of *SCN3A*. The patient was diagnosed as LGS. The patient responded well to an SCB, which is consistent with previous reports on patients with *SCN3A* variants related to GoF.[22] SCBs inhibit the transmission of action potentials through voltage-gated sodium channels, explaining their therapeutic efficacy against GoF variants of sodium-channel genes.[26,27] It was particularly interesting that our patient was able to maintain seizure freedom even after all ASMs except CBD were tapered out. CBD is reported to block the sodium channel by binding close to the central pore just below the sodium ion selectivity filter, which inhibits sodium-channel activity, as demonstrated by voltage-clamp studies of transfected cells.[28]

SCN4A encodes the alpha subunit of the voltage-gated sodium channel, which is widely expressed in skeletal muscles. This channel is involved in the excitability and contraction of muscle membranes. However, Nav1.4 may also be expressed in the brain.[29] Several studies have explored missense variants of *SCN4A* related to neuromuscular phenotypes, more

of which are GoF than LoF variants.[30] Some of these patients were reported to experience generalized seizures. Duan et al.[31] reported a patient with a missense variant of *SCN4A* diagnosed with alternating hemiplegia and epilepsia partialis continua. The c.2023C>T variant identified in our study was previously reported to be associated with potassium-sensitive normokalemic periodic paralysis.[14] This missense variant was located in segment S4 of domain II, which is the voltage-sensing domain.[14] Our proband showed refractory EIDEE with myoclonic absence seizures, and all of the administered SCBs were ineffective. Effective ASMs were PGB and TPM, which act on high-voltage-activated calcium channels.[32] The Nav1.4 channel has a unique calmodulin-mediated calcium ion dependent inactivation mechanism that leads to a decrease in the sodium ion current when the calcium ion level increases, which explains the efficacy of these CCBs in our proband.[33]

KCNA1 encodes the delayed-rectifier potassium channel Kv1.1 that is expressed by inhibitory neurons in the brain. This potassium channel controls neuronal excitability by enabling repolarization after an action potential and by influencing the resting membrane potential.[34] Missense LoF variants of *KCNA1* are associated with various types of epilepsy including DEE, episodic ataxia, myokymia, musculoskeletal abnormalities, and nystagmus.[35,36] Epilepsy phenotypes were associated with the pore-forming transmembrane domain of the protein, which was also the case in our patient. Although the c.1112C>T variant was previously reported by our institution, here we have additionally specified its clinical characteristics.[15] Our proband was diagnosed with LGS and showed a >50% reduction in seizure frequency following treatment with a SCB and the ketogenic diet. SCBs may be therapeutically effective by decreasing the overall threshold of action potentials and enhancing the inactivated state. Furthermore, SCBs were also found to increase the minimum electrical current required to initiate an action potential in pyramidal cells with LoF variants of *KCNA1*, which were identified in episodic ataxia patients.[37] Previous clinical studies of epilepsy patients with missense LoF variants of *KCNA1* have also found seizure reduction with SCBs such as OXC and LCS.[38,39] In addition, the therapeutic efficacy of ketogenic diet has been confirmed in studies of *KCNA1*-null mice. In these mice, the ketogenic diet restored certain histopathological changes in the CA1 hippocampus, which resulted in a decrease in seizure burden.[40]

KCNA2 encodes the shaker-like delayed-rectifier potassium channel Kv1.2. The gene is expressed in inhibitory neurons. Kv2.1 can inhibit the rapid firing of neurons.[41] Seizures and reduced lifespan were observed in mice lacking Kv1.2.[42] Both GoF and LoF variants of *KCNA2* cause a broad spectrum of neurological disorders, including focal epilepsy to generalized seizures (sometimes refractory), and developmental delay, spastic paraplegia, episodic ataxia, and cerebellar ataxia.[43] In the present study we identified three pathogenic variants of *KCNA2*: the c.785C>T variant was located in segment S3, whereas the c.1250A>G and c.1130A>G variants were located between segments S5 and S6, which were previously described as mutational hotspots.[16,44] Döring et al.[16] described a patient with the c.1250A>G variant experiencing benign epilepsy during infancy who achieved seizure freedom after 10 months with normal EEG findings and normal development. In contrast, in our study the older sister with the c.1250A>G variant showed an older age at seizure onset, at 10 years old. The c.785C>T and c.1130A>G variants were reported previously by our institution, but not their specific clinical characteristics.[17] The probands were diagnosed with focal epilepsy but manifested with generalized seizures. The proband with the c.785C>T variant also exhibited developmental delay and an ataxic gait.

All of our patients eventually showed seizure freedom. Seizures were reduced significantly by a SCB, consistent with previous findings of 8 of 14 patients with *KCNA2* mutations responding to SCBs.[45] Niday and Tzingounis[46] postulated that in non-inactivated GoF variants of *KCNA2*, partially inactivated Kv1.2 channels would lead to the maintenance of axonal sodium channel availability, resulting in faster neuronal firing. This neuronal firing may be blocked by SCBs. In addition, SCBs may be effective for seizure control in patients with GoF variants of *KCNA2* due to a hyperpolarized membrane facilitating the reactivation of voltage-gated sodium channels.[46] This explains the mechanism by which SCBs decrease the firing threshold and widen the action potentials, thereby ultimately lowering the seizure threshold.[47]

KCNB1 encodes the alpha subunit of the delayed-rectifier potassium channel Kv2.1, which is involved in cortical pyramidal neuron excitability and neurotransmitter release. Kv2.1 channels are expressed in both excitatory and inhibitory neurons found in the hippocampus, cortex, and dorsal root ganglia.[48,49] Affected patients show a broad spectrum of neurodevelopmental disorders, including DEE, autism spectrum disorders, and psychiatric disorders.[48] In concordance with previous studies, five of our six patients with *KCNB1* mutations were diagnosed with DEE and were refractory to multiple ASMs. Half of the patients were diagnosed with LGS. The c.934C>T variant that was previously reported as being associated only with developmental delay was associated with both LGS and focal epilepsy in our study.[19] The patients carrying the c.1106G>T and c.1237G>A variants were diagnosed with LGS.[17]

While previously reported EEG findings have varied widely

among patients carrying *KCNB1* variants, two of our six patients showed continuous spikes and waves during slow-wave sleep. Previous reports have indicated the therapeutic efficacy of LEV, VPA, and the ketogenic diet in these patients.[19,48,50,51] Similarly, two of our three patients who tried the ketogenic diet showed significant seizure reductions. In addition, five (83%) of six patients also responded to VPA treatment. Notably, three (75%) of four patients treated with CLB also showed seizure reduction. Given that mutations in *KCNB1* may alter both NMDA and GABA transmission, enhancing inhibitory GABA A receptor activation by administering GABAergic ASMs (i.e., VPA, CLB, or the ketogenic diet) may reduce seizures.[52,53]

KCNC1 encodes the Kv3.1 channel, which is responsible for the rapid activation and deactivation of fast-spiking inhibitory neurons in response to depolarization.[54] Kv3.1 is expressed in various parts of the brain, including the neocortex, hippocampus, and cerebellum.[55] Zhang et al.[56] suggested that epileptogenesis occurs with *KCNC*1 mutations due to a decreased ability of cortical inhibitory neurons to fire rapidly. The phenotypes include epilepsy in infancy with migrating focal seizures, progressive myoclonic epilepsy, epileptic encephalopathy, ataxia, and intellectual disability.[56-58]

Our patient carried a pathogenic missense variant, previously found in six patients with DEE carrying *KCNC1* mutations.[21] Similar to previous reports, our patient experienced myoclonic seizures and was also diagnosed with DEE. VPA was effective in our patient, similar to the patients reported by Cameron et al.[21] A possible hypothesis for this therapeutic efficacy is that VPA induces a reduction in the number of parvalbumin-positive cells and decreased parvalbumin mRNA. More specifically, the expression of *KCNC1* in these parvalbumin neurons was significantly decreased in VPA-exposed mice.[59] Parvalbumin is associated with epileptogenesis, since selective activation of parvalbumin interneurons is reported to be critical for the generation of epileptiform discharges in the entorhinal cortex of mice.[60] Thus, VPA may alter neuronal signaling and parvalbumin expression in a gene-specific mechanism, specifically for *KCNC1*.

KCNMA1 encodes the alpha subunit of the BK channel, which is a large-conductance, voltage-gated, and calcium-sensitive potassium channel expressed in the brain and muscles that plays a critical role in the regulation of neuronal excitability by speeding up the repolarization of action potentials.[61,62] Our proband carried the missense variant c.1807A>G, which had only been reported in ClinVar, but without a specific description. This variant is located after the S6 segment in the intracellular C-terminus of the protein, which is sensitive to calcium ions. The GoF and LoF variants of *KCNMA1* share certain phenotypes, such as epilepsy with various seizure types, developmental delay, and movement disorders.[63,64] GoF variants are mostly missense variants and show paroxysmal nonkinesigenic dyskinesia. LoF variants lead to ataxia, tremors, and Liang-Wang syndrome, all of which cause visceral malformations and facial dysmorphisms.[65]

Our proband showed both myoclonic and focal impaired-awareness seizures along with developmental delay and ataxia. The missense variant of our proband was suspected to be a GoF variant due to the lack of visceral and facial malformations. Moreover, the patient responded to the R-type CCB TPM, which may support the GoF hypothesis in our proband. The therapeutic efficacy of TPM in our patient was supported by a previous report that verapamil, an L-type CCB, inhibits BK channels at the cellular level in the aortic myocytes of rat models.[66] Furthermore, the pathogenic variant in our study was located near the C-terminus, which is more sensitive to calcium ions.

This study has contributed to the understanding of the genotypic and phenotypic spectra of less-common sodium and potassium channelopathies associated with epilepsy. Effective treatments for these channelopathies have also been described. However, this study had several limitations. In addition to the absence of functional studies of the variants, the number of included patients was small. The retrospective design also meant that bias might have affected the effects of the different treatments. The pharmacological mechanism of action of ASMs needs to be clarified through analyses of experimental in vitro and in vivo models.

Further research is needed to confirm the various electrophysiological effects that each pathogenic variant can have on ion channels. More clinical data must be accumulated to determine a more-accurate phenotype of these channelopathies. Such information might make it possible to provide tailored treatments to patients, including the repurposing of existing drugs and novel genetic therapies.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

ORCID iDs

Hui Jin Shin	https://orcid.org/0000-0002-1541-2564
Ara Ko	https://orcid.org/0000-0002-3008-8432
Se Hee Kim	https://orcid.org/0000-0001-7773-1942
Joon Soo Lee	https://orcid.org/0000-0001-9036-9343
Hoon-Chul Kang	https://orcid.org/0000-0002-3659-8847

Author Contributions

Conceptualization: Hui Jin Shin, Hoon-Chul Kang. Data curation: Ara Ko, Se Hee Kim, Joon Soo Lee, Hoon-Chul Kang. Formal analysis: Hui Jin Shin, Ara Ko. Funding acquisition: Hoon-Chul Kang. Investigation: Hui Jin Shin, Ara Ko, Se Hee Kim, Joon Soo Lee. Methodology: Hui Jin Shin, Ara Ko. Project administration: Hoon-Chul Kang. Resources: Ara Ko, Se Hee Kim, Joon Soo Lee, Hoon-Chul Kang. Software: Hui Jin Shin, Ara Ko.

Supervision: Ara Ko, Hoon-Chul Kang. Validation: Hui Jin Shin, Ara Ko. Visualization: Hui Jin Shin. Writing—original draft: Hui Jin Shin. Writing—review & editing: Ara Ko, Hoon-Chul Kang.

Conflicts of Interest

Hoon-Chul Kang, a contributing editor of the *Journal of Clinical Neurology*, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

Funding Statement

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2022R1A2C1012522); a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (grant number: HI21C1659); and the Team Science Award of Yonsei University College of Medicine (6-2021-0007).

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