Dominant *KCNA2* mutation causes episodic ataxia and pharmacoresponsive epilepsy

Mark A. Corbett, PhD
Susannah T. Bellows,
MPsych
Melody Li, PhD
Renée Carroll, BSc
Silvana Micallef, PhD
Gemma L. Carvill, PhD
Candace T. Myers, PhD
Katherine B. Howell,
MBBS
Snezana Maljevic, PhD
Holger Lerche, MD
Elena V. Gazina, PhD
Heather C. Mefford, MD,
PhD
Melanie Bahlo, PhD
Samuel F. Berkovic, MD
Steven Petrou, PhD
Ingrid E. Scheffer, MBBS,
PhD*
Jozef Gecz, PhD*

Correspondence to
Dr. Gecz:
jozef.gecz@adelaide.edu.au
or Dr. Scheffer:
scheffer@unimelb.edu.au

ABSTRACT

Objective: To identify the genetic basis of a family segregating episodic ataxia, infantile seizures, and heterogeneous epilepsies and to study the phenotypic spectrum of *KCNA2* mutations.

Methods: A family with 7 affected individuals over 3 generations underwent detailed phenotyping. Whole genome sequencing was performed on a mildly affected grandmother and her grandson with epileptic encephalopathy (EE). Segregating variants were filtered and prioritized based on functional annotations. The effects of the mutation on channel function were analyzed in vitro by voltage clamp assay and in silico by molecular modeling. *KCNA2* was sequenced in 35 probands with heterogeneous phenotypes.

Results: The 7 family members had episodic ataxia (5), self-limited infantile seizures (5), evolving to genetic generalized epilepsy (4), focal seizures (2), and EE (1). They had a segregating novel mutation in the *shaker* type voltage-gated potassium channel *KCNA2* (CCDS_827.1: c.765_773del; p.255_257del). A rare missense *SCN2A* (rs200884216) variant was also found in 2 affected siblings and their unaffected mother. The p.255_257del mutation caused dominant negative loss of channel function. Molecular modeling predicted repositioning of critical arginine residues in the voltage-sensing domain. *KCNA2* sequencing revealed 1 de novo mutation (CCDS_827.1: c.890G>A; p.Arg297Gln) in a girl with EE, ataxia, and tremor.

Conclusions: A *KCNA2* mutation caused dominantly inherited episodic ataxia, mild infantile-onset seizures, and later generalized and focal epilepsies in the setting of normal intellect. This observation expands the *KCNA2* phenotypic spectrum from EE often associated with chronic ataxia, reflecting the marked variation in severity observed in many ion channel disorders. *Neurology*® 2016;87:1975-1984

GLOSSARY

EA = episodic ataxia; **EE** = epileptic encephalopathy; **GATK** = genome analysis toolkit; **GGE** = genetic generalized epilepsies; **GTCS** = generalized tonic-clonic seizures; **ICCA** = infantile convulsions choreoathetosis syndrome; **RMS** = root mean square.

De novo mutations of *KCNA2* have recently been reported in infantile-onset epileptic encephalopathies (EEs) in which infants present with uncontrolled seizures, developmental slowing or regression, and frequent epileptiform activity on EEG.¹⁻⁶ Inherited mutations have not been described. *KCNA2* has not been implicated in common pharmacoresponsive epilepsies such as the genetic generalized epilepsies (GGE), nor has an association with paroxysmal movement disorders been established.

Paroxysmal neurologic disorders are often due to disorders of ion channel function. The episodic ataxias (EAs) are usually dominantly inherited and associated with a range of ion channel

Supplemental data
at Neurology.org

*These authors contributed equally to this work.

From the School of Medicine and Robinson Research Institute (M.A.C., R.C., J.G.) and School of Biological Sciences (J.G.), The University of Adelaide; Epilepsy Research Centre, Department of Medicine (S.T.B., S. Micallef, S.F.B., I.E.S.), University of Melbourne, Austin Health, Heidelberg; Florey Institute of Neuroscience and Mental Health (M.L., S. Maljevic, E.V.G., S.P., I.E.S.), Melbourne; Division of Genetic Medicine, Department of Pediatrics (G.L.C., C.T.M., H.C.M.), University of Washington, Seattle; Department of Neurology (K.B.H., I.E.S.), Royal Children's Hospital; Neurosciences Group (K.B.H.), Murdoch Childrens Research Institute, Melbourne; Department of Paediatrics (K.B.H.), University of Melbourne, Royal Children's Hospital, Parkville, Australia; Department of Neurology and Epileptology (S. Maljevic, H.L.), Hertie Institute for Clinical Brain Research, University of Tübingen, Germany; The Walter and Eliza Hall Institute of Medical Research (M.B.); Department of Medical Biology (M.B.), The University of Melbourne, Parkville; and Department of Medicine, Royal Melbourne Hospital (S.P.), The University of Melbourne, Australia.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

genes including the potassium channel gene *KCNA1* (OMIM: 160120)⁷ and the calcium channel gene *CACNA1A* (OMIM: 601011).⁸ Here, we expand the repertoire of genes for the EAs, particularly when associated with seizures.

Voltage-gated potassium (K⁺) channels are encoded by 40 genes representing 12 protein subfamilies K_v1–K_v12.⁹ The K_v1 or *shaker* subfamily channels comprise heterotetramers or homotetramers of α-subunits that have 6 α-helical transmembrane domains (S1–S6).¹⁰ The transmembrane domains form the voltage sensor (S1–S4) and the surface of the channel pore (S5 and S6). The K_v1 family channels are broadly expressed; however, individual isoforms are expressed in different combinations in different tissues.¹¹ K_v1.1, K_v1.2, and K_v1.4 are the most highly expressed subunits in the CNS.¹¹ *KCNA2* encodes an α-subunit of the K_v1.2 channel. Mice null for *Kcna2* have a shortened lifespan, seizures, and ataxia.¹²

Using whole genome sequencing, we discovered an in-frame deletion in *KCNA2* (CCDS_827.1: c.765_773del;p.255_257del)

that segregated with a dominant epilepsy-EA syndrome with marked variation in severity within the family.

METHODS Cohort selection and phenotyping. The WF family, comprising 4 affected individuals over 3 generations (figure 1A), was initially referred with a history of autosomal dominant infantile-onset seizures and a movement disorder.¹³ Since publication, 3 new affected children have been born and the phenotype of the proband has evolved, extending the spectrum of their familial syndrome to include focal epilepsy and EE. Two individuals (WF-I-1, WF-III-6) underwent whole genome sequencing.

KCNA2 was then sequenced in 35 patients with EA alone (1), EA and epilepsy (1), other paroxysmal movement disorders with seizures (n = 7) and without (n = 7), epilepsy and chronic ataxia (n = 2), and infantile seizures alone (n = 17). Participants were recruited from epilepsy clinics, private practices, and by referral to our epilepsy and related conditions genetics research program. Detailed phenotypic information was obtained from participants and family members using a validated seizure questionnaire.¹⁴ All medical records, EEG, and neuroimaging data were obtained where available.

Standard protocol approvals, registrations, and patient consents. Informed written consent was obtained from participants or their parents in the case of minors. The study was approved by the Austin Health Human Research Ethics Committee, Australia.

Whole genome and whole exome sequencing. Whole genome sequencing (150 bp, paired end) was performed using

Figure 1 Family with an autosomal dominant *KCNA2* mutation associated with epilepsy and episodic ataxia

(A) WF family pedigree. The *KCNA2* mutation (*) segregates with affected status in all individuals. The *SCN2A* variant (#) is inherited from the unaffected mother. (B) A 9-base pair heterozygous deletion identified in WF-I-1 and WF-III-6. Alignment of whole genome sequencing reads against hg19 genome as seen in the integrative genome viewer.⁴⁰ The translation of the wild-type and mutant alleles is indicated with single letter amino acid codes below the alignment.

an Illumina (San Diego, CA) X-10 platform (Kinghorn Centre for Clinical Genomics, Sydney, Australia). Whole exome sequencing (100 bp, paired-end) was performed by the Australian Genomics Research Facility using sequence capture targets from the SureSelectXT Exome enrichment kit V5 (Agilent Technologies, Santa Clara, CA). Reads were mapped to the human genome build (hg19) with BWA-MEM using default options.¹⁵ Alignments were refined with local realignment around indels and variants called and refined using the genome analysis toolkit (GATK) v3.2-2.¹⁶ Variants that passed GATK quality filters and were shared between affected individuals were identified using vcftools. Systematic variant calling errors and common variants were removed using vcf contrast against a set of variants found in at least 3 of 8 other unrelated genomes called at the same time. The remaining variants were annotated with ANNOVAR prior to further filtering.¹⁷ Common variants with a minor allele frequency of greater than 1:1000 in ExAC, Exome Variant Server and UK10K data greater than 1:200 in 1000 genomes project and Complete Genomics Welllderly data were excluded. Variants were prioritized by (1) removing noncoding and synonymous variants not predicted to affect splicing; (2) removing variants in genes that did not fit with known phenotypes in OMIM and the Deciphering Developmental Disorders study¹⁸; and (3) removing variants in genes not expressed in brain based on GTEX RNA sequencing data ($\log_{10}$ RPKM values <0).¹¹

Sanger sequencing. Variants in *KCNA2* were verified from PCR amplicons of genomic DNA by Sanger sequencing (table e-1 at Neurology.org shows primer sequences).

Voltage clamp assay. We obtained the plasmid containing human *KCNA2* cDNA in pcDNA3.1/Hygro vector¹⁹ and the c.765-773 deletion was introduced into *KCNA2* using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies) following the manufacturer's instructions. Construct fidelity was confirmed by DNA sequencing. cRNAs were transcribed in vitro using T7 mMessage mMachine Kit (Ambion; Thermo Fisher Scientific, Waltham, MA).

Oocytes (stage V–VI) were isolated from *Xenopus laevis* as described previously.²⁰ Oocytes were injected with 50 ng of each cRNA using a Roboinject (Multichannel Systems, Reutlingen, Germany). After 2 days of incubation, 2-electrode voltage clamp recording was performed on a Roboocyte2 (Multichannel Systems). Electrodes contained 1.5 M $\text{KC}_2\text{H}_3\text{O}_2$ and 0.5 M KCl and oocytes were held at -80 mV. Experiments were performed in ND96, which contained 96 mM NaCl, 2 mM KCl, 1 mM MgCl_2 , 1.8 mM CaCl_2 , and 5 mM HEPES, pH 7.5. Current was recorded using P/4 correction with a series of 600-ms steps from -60 to $+40$ mV in 10-mV increments with a 2-second interval between steps. Data were recorded at 2 kHz and temperature maintained at 22°C – 24°C .

Modeling of protein structure. Wild-type and mutant *KCNA2* sequences were submitted to the I-TASSER server²¹ and predictions of protein structure made against existing models using default measures. Images were generated from predicted structures that had the highest C-score with Swiss PDB viewer v4.04.²² Calculation of root mean square (RMS) differences between mutant and wild-type carbon alpha chains was performed by selecting the most displaced amino acids in the region surrounding the deletion in both the mutant and wild-type proteins and using the “Calculate RMS...” option in Swiss PDB viewer v4.04.²²

RESULTS Phenotypic analysis. Family WF. The family comprised 7 affected individuals over 3 generations (family WF)¹³ (figure 1A and table 1). All 7

individuals had epilepsy and 5 had EA. The family included 3 affected adults with EA including 2 with GGE. Six of the 7 individuals had infantile-onset seizures, 3 evolving to GGE alone, GGE and focal epilepsy in 1, and a severe EE (table 1).

Infantile onset of seizures occurred at a median of 7 months (6–15 months) with clusters of generalized tonic-clonic seizures (GTCS). The 54-year-old father (WF-II-3) had rare GTCS thereafter until 44 years. The cousin (WF-III-7) was seizure-free from 9 months until 8 years, when occipital seizures developed. Absence seizures associated with 3-Hz generalized spike-wave were recorded at 9 years with rare GTCS thereafter. The proband WF-III-4 had GTCS until 5 years, with 2 later GTCS at 19 and 20 years associated with medication noncompliance. WF-II-4 had 4 GTCS with a single afebrile convulsion at 15 months and 3 between 19 and 24 years. Two individuals had childhood absence epilepsy, with the proband evolving to juvenile myoclonic epilepsy. All 4 individuals with GGE had generalized spike-wave activity with photic sensitivity; the cousin (WF-III-7) also had occipital sharp waves. The proband's brother (WF-III-5) had focal epilepsy beginning at age 13 years with a perceptual aura of the environment “going fast around him.” He had 2 tonic-clonic seizures at 16 and 19 years. The proband, her brother, and her cousin all had delayed speech development, with first words between 18 months and 3 years. The siblings WF-III-4 and WF-III-5 had low average intellect in late childhood and their cousin WF-III-7 had mild intellectual disability (full-scale IQ 69) and high functioning autism. Development in the father, aunt, and grandmother was normal.

Five individuals had EA beginning at mean 8 years (median 9 years, range 5–12 years). It was characterized by loss of coordination in the lower limbs, often associated with dysarthria.

Individuals reported a vague warning before onset of an episode, retained awareness during the event, and dysarthria often preceding and persisting beyond involvement of limbs. No myokymia or nystagmus was observed. Episodes often occurred in clusters and varied in duration from brief attacks lasting 15 seconds to prolonged episodes lasting up to 12 hours. The events were triggered by exercise, fatigue, illness, menstruation, startle, stress, and sudden movement. Acetazolamide was effective in reducing episodes in 2 individuals.

Individual WF-III-6, aged 14 years, has a severe epileptic encephalopathy. He had normal development until tonic-clonic seizures began at 7 months, and frequent photic induced absence seizures were observed by 13 months. Myoclonus was present at 18 months, with multifocal myoclonus evident by 6 years. He had frequent nonconvulsive status

Table 1 Clinical summary of individuals with new *KCNA2* mutations

Family member	Sex	Current age, y	Episodic ataxia onset (frequency) [duration] triggers	Epilepsy	Age at onset	Age at last seizure	Seizure type (onset)	Total no. of TCS	Frequency	Response to medication	EEG studies	Photosensitive	MRI results	Development and intellect	Other features
WF-I-1	F	81	Early childhood (1/mo) [30 s–5 min] stress	Infantile seizures	Infantile	NA	TCS	NA	NA	NA	NA	NA	Atrophy of cerebellar vermis	Normal	Mildly antalgic gait
				Unclassified epilepsy	4 y	76 y	TCS	7	1–2/y, seizure-free from childhood until 40 y, and then 40 y–74 y	Responsive to LEV and DHT	61 y: normal	NA			
WF-II-3	M	54	10 y (1/w–3/y) [15 s–1 min] fatigue, exercise, illness, stress, sudden movement	Infantile seizures	7 mo	7 y	TCS	>20	Clusters of TCS, 4–5/mo	Responsive to PHT, no response to PB	5 y: GSW, PSW	Y	NA	Normal	
				GGE	7	46 y	Nocturnal TCS, Abs	>20	1–2/y, Rare Abs	Responsive to PHT and VPA	47 y: 3-Hz GSW	N			
WF-II-4	F	48	5 y (rare) [10 min] exercise, stress	Infantile seizures	15 mo	15 mo	TCS	1	Single TCS				Normal	Normal	
				CAE	4 y	24 y	Abs (4 y), TCS (19 y)	3	8/d Abs, no TCS 15 mo–19 y	Responsive to VPA and CBZ	28 y: 2–3 Hz GSW	Y			
WF-III-4	F	21	12 y (5–6/y) decreased with acetazolamide [1–2 min] exercise, fatigue, illness, menstruation, stress, startle	Infantile seizures	7 mo	5 y	TCS	>10	Cluster of TCS at onset	Responsive to CBZ	2 y: PSW	Y	Normal	Delayed speech, low average	
				CAE-JME	9 y	20 y	Abs (9 y), MJ (12 y), TCS (19 y)	2	Multiple/d Abs, infrequent TCS	Responsive to VPA	12 y: GSW, PSW	Y			
WF-III-5	M	19	9 y (multiple/d) decreased with acetazolamide [30–60 s–12 h] exercise, fatigue, illness, startle, stress	FE	13 y	19 y	FAS (13 y), TCS (16 y)	2	Rare FAS: perceptual changes: environment going fast around him	Responsive to CBZ and then VPA	9 y, 12 y: bifrontal discharge, R>L	N	Normal	Delayed speech, low average	
WF-III-6	M	15	NA	EE	7 mo	Ongoing	TCS (7 mo), SE (7 mo), Abs (13 mo), MJ (18 mo), NCSE (6 y)	>100	Frequent TCS in infancy, 50–100 absences/d (photic-induced), palpable multifocal myoclonus, no TCS 3.5 y–7 y, frequent SE and NCSE from 7 y	Refractory to VPA, ETX, stiripentol, CLB; CLB improved Abs and learning; honeymoon period with stiripentol introduction with photic induced Abs only; trialled CBZ, LEV, LTG, zonisamide	10 mo: normal; 2 y: frequent 2–2.5 GSW, PSW; 6 y 3 Hz PSW intermixed with fast activities	Y	Normal	Initially normal; slowed 10 mo; regression 18 mo; severe ID	Dystonia, ataxia

Continued

Table 1 Continued

Family member	Sex	Current age, y	Episodic ataxia onset (frequency) [duration] triggers	Epilepsy	Age at onset	Age at last seizure	Seizure type (onset)	Total no. of TCS	Frequency	Response to medication	EEG studies	Photosensitive	MRI results	Development and intellect	Other features
WF-III-7	M	13	NA	Infantile seizures	6 mo	9 mo	TCS	9	Cluster of TCS at onset	Responsive VPA	7 mo sharp SW occipital bilaterally	NA	Focal area of gliosis abutting right lateral ventricle in posterior frontal lobe	Delayed speech, mild ID (FSIQ 69), ASD	
				FE	8 y	10 y	FUS (8 y)		Visual aura preceding loss of awareness and limb automatisms	Responsive to LTG	3 Hz GSW; solitary sharp transients left occipital and right central region				
				GGE	9 y	12 y	Abs (9 y), TCS (10 y)	5	Abs noted on EEG at 9 y; TCS 1-2/y	Responsive to VPA and LTG		Y			
Proband B	F	9	NA	Epilepsy and ataxia	12 mo	Ongoing	T (12 mo), MJ (2 y), Abs (5 y)	NA	Tonic 3-4/y; erratic myoclonic tremor	Ongoing T seizures; Abs controlled VPA, LTG, CLB	14 mo: normal; 2 y: frequent irregular 2-3.5 Hz generalized SW and polyspike-wave in runs of 20-30 seconds, varies in anterior-posterior emphasis; 4 y: 3-4 Hz bilateral posterior SW, background slowing; 7 y: frequent bilateral posterior 3-4 Hz SW, brought out by eye closure, clinical absence seizures in hyperventilation with 3-Hz generalized SW	NA	2 y: normal; 8 y: mild cerebellar atrophy	Initially normal; slowed at 12 mo	Ataxia; cerebellar signs on examination; normal eye movement

Abbreviations: Abs = absences; ASD = autism spectrum disorder; CAE = childhood absence epilepsy; CBZ = carbamazepine; CLB = clobazam; DHT = dihydroxytryptamine; EE = epileptic encephalopathy; ETX = ethosuximide; FAS = focal aware seizures; FE = focal epilepsy; FSIQ = full-scale IQ; FUS = first unprovoked seizure; GGE = genetic generalized epilepsy; GSW = generalized spike-and-wave; ID = intellectual disability; JME = juvenile myoclonic epilepsy; LEV = levetiracetam; LTG = lamotrigine; MJ = myoclonic jerks; NA = not applicable; NCSE = nonconvulsive status epilepticus; PB = phenobarbital; PHT = phenytoin; PSW = polyspike-and-wave; SE = status epilepticus; SW = spike-wave; T = tonic; TCS = tonic-clonic seizures; VPA = valproate.

Figure 2 KCNA2 p.255_257del mutation results in a dominant negative loss of channel function

(A) Representative current traces from *Xenopus laevis* oocytes injected with water (gray) or cRNA encoding the wild-type (WT) (black), KCNA2 c.765_773del (dark blue), or a mixture of both (light blue) (B). Current amplitudes plotted over test pulses of -60 mV to 40 mV for oocytes injected with water (dotted line) or cRNA encoding wild-type KCNA2 (black), KCNA2 c.765_773del (dark blue), or a mixture of both (light blue).

epilepticus requiring admission and routinely had 80 absence seizures per day. He was refractory to multiple antiepileptic medications. He had severe intellectual disability and a crouch gait. Generalized spike-wave and polyspike-wave with photic sensitivity was seen on EEG.

Proband B. Proband B, with a de novo *KCNA2* mutation (see below), had onset of tonic seizures with pallor at 12 months. Seizures were initially triggered by minor head injuries and later occurred with infection, fever, or on awakening. Absence seizures commenced at 5 years following withdrawal of lamotrigine. Erratic myoclonic tremor began at 2 years following commencement of valproate but did not worsen with lamotrigine; this was exacerbated by illness, fatigue, stress, and on awakening for 1.5 hours (videos). Her gait was mildly ataxic; paroxysmal ataxia was not observed. There was no family history of tremor and a bilineal distant family history of epilepsy. Her development was initially normal and slowed with seizure onset. Neuropsychological assessment at 9 years showed significant verbal (average range)–nonverbal (extremely low range) discrepancy in the setting of an overall borderline IQ; this was not wholly explained by her difficulties with movement and tremor. EEG was normal at 14 months. At 2, 4, and 7 years, background activity was slow. Frequent

long runs of bisynchronous occipital spike-wave were seen, particularly with eye closure and drowsiness but not with photic stimulation. Generalized spike-wave and rare focal discharges were also seen.

Molecular analysis. Whole genome sequence reads from the grandmother WF-I-1 and her grandson WF-III-6 were mapped to the human genome hg19 and achieved a median depth of 29 reads with minimum mapping and base quality Phred scores of 20. There were 1,565,560 variants shared between genomes. After filtering variants that were unlikely to be disease-causing based on population frequency and prediction of functional effects, we found 4 candidate genes (table e-2). Of these, a deletion in *KCNA2* (CCDS_827.1: c.765_773del; p.255_257del) (figure 1B) was favored because both knockout and ENU-induced *Kcna2* mutant mice exhibit seizures and ataxia^{12,23} and de novo *KCNA2* mutations cause EE.^{1–5} The remaining 3 genes in which variants were found (*EGR4*, *MET*, *RNF19B*) have not been implicated in epilepsy or ataxia (table e-2). The *KCNA2* variant segregated with all affected individuals (figure 1B).

We performed whole exome sequencing of both parents of WF-III-6 and sought de novo, X-linked, potentially autosomal recessive, and maternally inherited variants in known or predicted epilepsy genes that might account for the increased severity of the phenotype in this individual (WF-III-6) compared to other family members. From these analyses, we identified 4 X-linked variants, 2 genes with compound heterozygous missense variants and 3 de novo variants, all of unknown effect (table e-3). We found only one variant that was inherited from the unaffected mother (WF-II-3) of WF-III-6 in a gene previously implicated in EE; *SCN2A* (NM_001040143: c.82C>T; p.Arg28Cys; rs200884216). It was also present in his sister WF-III-4 but not in other affected family members. This variant was predicted to be damaging by several algorithms, is found in the general population at low frequency (ExAC 2.21×10^{-4} , table e-3), and could potentially modify the phenotype in WF-III-6.

We screened the *KCNA2* open reading frame and identified a recurrent de novo mutation (c.890G>A; p.Arg297Gln) in 1/35 individuals (proband B) in our cohort.^{1,2} p.Arg297Gln neutralizes the second arginine in the voltage sensor in $K_v1.2$. Voltage clamp assays demonstrated a 9-fold increase in current amplitude and a shift of voltage-dependent activation by -40 mV compared with wild-type, also observed for a mixture of WT and mutant channels, thus consistent with a dominant, strong gain of function.²

To test the effect of the familial *KCNA2* c.765_773del mutation on channel function, we expressed the mutant, wild-type, and a combination of both in *Xenopus laevis* oocytes. Oocytes expressing

(A) A portion of a CLUSTALW alignment of full-length KCNA2 orthologs from multiple vertebrate species. The 3 amino acids that are deleted from KCNA2 (indicated by the braces) are completely conserved in the mammalian, avian, amphibian, and fish species shown. Sequences were identified using the homologue database. (B) Prediction of secondary structures in the S3 domain of wild-type and mutant KCNA2 shows an increase in the α -helical content (H) compared to coil (C) structures. (C) The predicted carbon α -chain structures of wild-type (left) and mutant (right) KCNA2. The region where the deleted amino acids are located is indicated by (*) and a significant deviation between the mutant and wild-type structures can be observed at this position. Critical basic and acidic residues of the voltage-sensing domain are highlighted in magenta. Arrowheads highlight the predicted repositioning of Arg294 and Arg309.

only wild-type *KCNA2* showed voltage-dependent activation with a peak current of $1.82 \pm 0.24 \mu\text{A}$ at +40 mV. In contrast, expression of *KCNA2* c.765_773del yielded significantly lower currents that peaked at $0.21 \pm 0.02 \mu\text{A}$ at +40 mV. We injected equivalent amounts of wild-type and *KCNA2* c.765_773del cRNAs and found clear evidence of dominant negative effect as peak current was $0.28 \pm 0.08 \mu\text{A}$ at +40 mV compared to wild-type alone (figure 2, A and B).

The *KCNA2* c.765_773del;p.255_257del mutation deletes residues that are identical in all mammalian, avian, amphibian and fish species examined (figure 3A). *KCNA2* p.Arg297 is invariable in mammals, fish, amphibians, and insects and is also highly conserved in the voltage-sensing domains of other ion channels.^{2,24}

We used molecular modeling to predict the effect of the p.255_257del mutation on the structure of KCNA2 (Protein Databank ID: 2R9R). These analyses predicted an increase in the α -helical content of S3, removing a turn in that helix that is usually juxtaposed with the cytoplasmic side of the plasma membrane (figure 3B). Analysis of the model 3D structure revealed a local 3.03 Å root mean square difference in the position of the mutant compared to the wild-type carbon α -chain backbone (figure 3C). The model also predicts altered positioning of critical residues Arg294 (R1) and Arg309 (R6) on the S4 helix of

the voltage-sensing domain (figure 3C). *KCNA2* shows remarkable coexpression with other genes implicated in EA (*KCNA1*, *CACNA1A*, *CACNB4*, *SLC1A3*; figure e-1).¹¹

DISCUSSION Seven mutations in *KCNA2* have been identified in 11 unrelated individuals with EE (figure e-2).¹⁻⁵ Here, we report the first autosomal dominant *KCNA2* disorder with a predominant phenotype of infantile-onset seizures with later GGE or focal epilepsy in the setting of normal intellect. We implicate *KCNA2* in EA for the first time. We also report a recurrent de novo mutation, highlighting a mutational hotspot (figure e-2).^{1,2}

Family WF considerably expands the phenotypic spectrum of *KCNA2* diseases to include mild well-controlled epilepsies and EA. All but one affected individual had infantile-onset seizures leading us initially to consider infantile convulsions choreoathetosis syndrome (ICCA),²⁵ although the movement disorder, EA, is clinically distinct from paroxysmal kinesigenic choreoathetosis. Their later evolution to GGE and focal epilepsy is also unusual for ICCA. *PRRT2* sequencing was negative in WF-III-4.²⁵

Many types of EA are recognized. EA1 is associated *KCNA1* mutation and focal epilepsy,²⁶ while EA2 due to *CACNA1A* mutations is associated with generalized epilepsy.²⁷ Mutational analysis of these genes was negative (*CACNA1A* in WF-II-4, *KCNA1* in WF-II-3).

Our findings strengthen the phenotypic overlap observed between *KCNA1* and *KCNA2*; the corresponding mutation in $K_v1.1$ p.Ile262Thr caused EA1 with distal weakness, while $K_v1.2$ p.Ile263Thr caused EE.^{2,28} We show that *KCNA2* is a cause of EA; further EA cohorts should be tested for *KCNA2* mutations.

The *KCNA2* p.255_257del mutation was fully penetrant in the family although there was a wide spectrum of phenotypic severity. As the boy WF-III-6 had a severe EE, we explored whether a second hit mutation could account for his far more severe phenotype by performing whole exome studies on his parents. We identified an *SCN2A* variant (NM_001040143: c.82C>T; p.Arg28Cys; rs200884216) that was predicted to be damaging, inherited from his unaffected mother and also present in his sister WF-III-4. Computer modeling indicates that multiple small changes in ion channel properties can lead to large changes in network excitability²⁹ and firing patterns of individual neurons.³⁰ Identifying and predicting which factors modify phenotypes remains a challenge for the future.

The EE in WF-III-6 and Proband B are congruent with 9 previous *KCNA2* mutation cases but differ markedly in severity.^{1–3} Proband B shared the constant myoclonus (videos) reported in other cases, together with absence seizures and ataxia. WF-III-6 had severe intellectual disability, about 100 absences daily, and frequent nonconvulsive status epilepticus.¹ However, it has to be considered whether WF-III-6's phenotype has been modified by the *SCN2A* variant.

The p.255_257del mutation is located in the third transmembrane domain S3, near the S2-S3 linker region.³¹ The deletion occurs adjacent to Asp259, which is a critical residue in the voltage-sensing domain.³¹ The predicted altered structure of the p.255_257del mutant protein could reduce protein stability. The mutation may also interfere with the function of the voltage-sensing domain, given the proximity of the mutation to Asp259 and the predicted repositioning of Arg294 and Arg309, which are conserved residues (R1 and R6) in most ion channels.²⁴ The dominant negative effect we measured by voltage clamp assay suggests that the *KCNA2* p.255_257del mutant can be incorporated into $K_v1.2$ channels. In tissues where there is a mix of K_v1 isoforms, the mutant *KCNA2* proteins could interact with other α -subunits expressed from *shaker* subfamily genes, potentially blocking their function.^{32,33}

KCNA2 diseases add to the emerging phenotypic spectrum characteristic of many ion channel disorders. For example, benign familial neonatal epilepsy is associated with mutations in *KCNQ2* and *KCNQ3*, in which autosomal dominant inheritance is usual and the outcome is excellent.^{34,35} In contrast, *KCNQ2*

encephalopathy is associated with more refractory epilepsy, cerebral palsy, and a devastating prognosis³⁶; de novo mutations are usual. Similarly, sodium channel diseases (e.g., *SCN1A*, *SCN2A*) cause both self-limited, pharmacoresponsive epilepsies in the setting of normal intellect and EEs. Although some phenotype-genotype correlations have been reported for these genetic diseases, often the phenotypic outcome associated with a specific variant is unpredictable.

There is increasing recognition of the overlap between the epilepsies and movement disorders and is seen frequently in the EEs. For example, *KCNA2* encephalopathy is associated with ataxia and dystonia,^{1,2} *SCN2A* encephalopathy with dystonia, choreoathetosis,³⁷ and EA,³⁸ and *SCN8A* encephalopathy with choreoathetosis, dystonia, and ataxia.³⁹ The diversity of movement disorders for each genetic encephalopathy expands as further cases are identified. These findings illustrate the critical importance of understanding the phenotypic diversity of a genetic disorder.

AUTHOR CONTRIBUTIONS

Mark A. Corbett: bioinformatics, drafted the manuscript. Susannah T. Bellows: phenotyping, drafted the manuscript. Melody Li: electrophysiology, contributed to writing the manuscript. Renée Carroll: primer design, PCR and Sanger sequencing, edited manuscript. Silvana Micallef: neuropsychology phenotyping, edited manuscript. Katherine B. Howell: phenotyping, contributed to writing the manuscript. Gemma L. Carvill: excluded known epilepsy genes. Candace T. Myers: excluded known epilepsy genes. Heather C. Mefford: excluded known epilepsy genes. Snezana Maljevic: electrophysiology, data interpretation. Holger Lerche: cloned the *KCNA2* constructs and designed electrophysiology experiments. Elena Gazina: cloned the *KCNA2* constructs. Melanie Bahlo: study design and contributed to writing the manuscript. Samuel F. Berkovic: study design, phenotyping, and contributed to writing the manuscript. Steven Petrou: designed and supervised the study, contributed to data interpretation and manuscript writing. Ingrid E. Scheffer: designed and supervised the study, performed phenotyping, contributed to data interpretation, and drafted the manuscript. Jozef Gecz: designed and supervised the study, contributed to data interpretation and manuscript writing.

ACKNOWLEDGMENT

The authors thank family WF for their participation in this project, the families of the cohort of 35 patients, eResearchSA for computing infrastructure support, and the Kinghorn Centre for Clinical Genomics. Web resources: Complete Genomics Welllderly data: stsiweb.org/wellderly; GTEx Portal: gtexportal.org/home; ExAC: exac.broadinstitute.org; OMIM: omim.org; UK10K (from the European Genome-Phenome Archive): ebi.ac.uk/ega/datasets/EGAD00001000740 and ebi.ac.uk/ega/datasets/EGAD00001000741.

STUDY FUNDING

This project was supported by NHMRC program grants 628952 (S.T.B., S.F.B., J.G., I.E.S., S.P.) and 1054618 (M.B.), NHMRC Research Fellowships 1041920 (J.G.) and 1002098 (M.B.), NHMRC Practitioner Fellowship 1104831 (I.E.S.), WCH foundation MS McLeod research fellowship (M.A.C.), Victorian Government's Operational Infrastructure Support Program, and Australian Government NHMRC IRIISS.

DISCLOSURE

M. Corbett, S. Bellows, M. Li, R. Carroll, S. Micallef, G. Carvill, C. Myers, K. Howell, S. Maljevic, H. Lerche, E. Gazina, H. Mefford,

and M. Bahlo report no disclosures relevant to the manuscript. S. Berkovic has served on scientific advisory boards for UCB and Janssen-Cilag; serves on the editorial boards of *Lancet Neurology* and *Epileptic Disorders* and the Advisory Board of *Brain*; may accrue future revenue on pending patent WO61/010176: Therapeutic compound that relates to discovery of PCDH19 gene as the cause of familial epilepsy with mental retardation limited to females; is one of the inventors listed on a patent held by Bionomics Inc. on diagnostic testing of using the *SCN1A* gene, WO2006/133508; has received speaker honoraria from UCB; has received unrestricted educational grants from UCB, Janssen-Cilag, and Sanofi-Aventis; and receives/has received research support from the National Health and Medical Research Council of Australia and NINDS. S. Petrou serves as Scientific Advisory Board Member for Paimomix Inc. and as Scientific Founder for EpiPM: a Clarus Ventures company, on the editorial boards of *Neurobiology of Disease* and *PLOS Genetics*, and is one of the inventors listed on a patent held by Bionomics Inc. on diagnostic testing using the *SCN1A* gene, WO2006/133508. I. Scheffer is funded by an NHMRC Practitioner Fellowship; serves on the editorial boards of *Neurology*® and *Epileptic Disorders*; may accrue future revenue on patent WO61/010176 and WO2006/133508; has received speaker honoraria or travel funding from Athena Diagnostics, UCB, GSK, and Transgenomics; and receives/has received research support from the National Health and Medical Research Council of Australia, CURE, March of Dimes, and NINDS. J. Géczy reports no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received March 23, 2016. Accepted in final form July 26, 2016.

REFERENCES

1. Pena SDJ, Coimbra RLM. Ataxia and myoclonic epilepsy due to a heterozygous new mutation in *KCNA2*: proposal for a new channelopathy. *Clin Genet* 2015;87:e1–e3.
2. Syrbe S, Hedrich UBS, Riesch E, et al. De novo loss- or gain-of-function mutations in *KCNA2* cause epileptic encephalopathy. *Nat Genet* 2015;47:393–399.
3. Allen NM, Conroy J, Shahwan A, et al. Unexplained early onset epileptic encephalopathy: exome screening and phenotype expansion. *Epilepsia* 2016;57:e12–e17.
4. Allou L, Julia S, Amsellem D, et al. Rett-like phenotypes: expanding the genetic heterogeneity to the *KCNA2* gene and first familial case of *CDKL5*-related disease. *Clin Genet Epub* 2016 Apr 7.
5. Hundallah K, Alenizi A, AlHashem A, Tabarki B. Severe early-onset epileptic encephalopathy due to mutations in the *KCNA2* gene: expansion of the genotypic and phenotypic spectrum. *Eur J Paediatr Neurol* 2016;20:657–660.
6. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* 2010;51:676–685.
7. Browne DL, Ganther ST, Nutt JG, et al. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, *KCNA1*. *Nat Genet* 1994;8:136–140.
8. Ophoff RA, Terwindt GM, Vergouwe MN, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca²⁺ channel gene *CACNL1A4*. *Cell* 1996;87:543–552.
9. Robbins CA, Tempel BL. Kv1.1 and Kv1.2: similar channels, different seizure models. *Epilepsia* 2012;53(suppl 1):134–141.
10. Jan LY, Jan YN. Voltage-gated potassium channels and the diversity of electrical signalling. *J Physiol* 2012;590:2591–2599.
11. Melé M, Ferreira PG, Reverter F, et al. Human genomics: the human transcriptome across tissues and individuals. *Science* 2015;348:660–665.
12. Brew HM, Gittelman JX, Silverstein RS, et al. Seizures and reduced life span in mice lacking the potassium channel subunit Kv1.2, but hypoexcitability and enlarged Kv1 currents in auditory neurons. *J Neurophysiol* 2007;98:1501–1525.
13. Singh R, Macdonell RA, Scheffer IE, Crossland KM, Berkovic SF. Epilepsy and paroxysmal movement disorders in families: evidence for shared mechanisms. *Epileptic Disord* 1999;1:93–99.
14. Reutens DC, Howell RA, Gebert KE, Berkovic SF. Validation of a questionnaire for clinical seizure diagnosis. *Epilepsia* 1992;33:1065–1071.
15. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754–1760.
16. Van der Auwera GA, Carneiro MO, Hartl C, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics* 2013;11:11.10.1–11.10.33.
17. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010;38:e164.
18. Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. *Nature* 2015;519:223–228.
19. Visan V, Fajloun Z, Sabatier JM, Grissmer S. Mapping of maurotoxin binding sites on hKv1.2, hKv1.3, and hKCa1 channels. *Mol Pharmacol* 2004;66:1103–1112.
20. Petrou S, Ugur M, Drummond RM, Singer JJ, Walsh JV. P2X7 purinoceptor expression in *Xenopus oocytes* is not sufficient to produce a pore-forming P2Z-like phenotype. *FEBS Lett* 1997;411:339–345.
21. Yang J, Zhang Y. I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Res* 2015;43:W174–W181.
22. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* 1997;18:2714–2723.
23. Xie G, Harrison J, Clapcote SJ, et al. A new Kv1.2 channelopathy underlying cerebellar ataxia. *J Biol Chem* 2010;285:32160–32173.
24. Börjesson SI, Elinder F. Structure, function, and modification of the voltage sensor in voltage-gated ion channels. *Cell Biochem Biophys* 2008;52:149–174.
25. Ebrahimi-Fakhari D, Saffari A, Westenberger A, Klein C. The evolving spectrum of PRRT2-associated paroxysmal diseases. *Brain* 2015;138:3476–3495.
26. Zuberi SM, Eunson LH, Spauschus A, et al. A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain* 1999;122:817–825.
27. Jouvenceau A, Eunson LH, Spauschus A, et al. Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. *Lancet* 2001;358:801–807.
28. Klein A, Boltshauser E, Jen J, Baloh RW. Episodic ataxia type 1 with distal weakness: a novel manifestation of a potassium channelopathy. *Neuropediatrics* 2004;35:147–149.
29. Thomas EA, Reid CA, Berkovic SF, Petrou S. Prediction by modeling that epilepsy may be caused by very small functional changes in ion channels. *Arch Neurol* 2009;66:1225–1232.
30. Klassen T, Davis C, Goldman A, et al. Exome sequencing of ion channel genes reveals complex profiles confounding personal risk assessment in epilepsy. *Cell* 2011;145:1036–1048.

31. Chen X, Wang Q, Ni F, Ma J. Structure of the full-length Shaker potassium channel Kv1.2 by normal-mode-based X-ray crystallographic refinement. *Proc Natl Acad Sci USA* 2010;107:11352–11357.
32. Sheng M, Liao YJ, Jan YN, Jan LY. Presynaptic A-current based on heteromultimeric K⁺ channels detected in vivo. *Nature* 1993;365:72–75.
33. Wang H, Kunkel DD, Martin TM, Schwartzkroin PA, Tempel BL. Heteromultimeric K⁺ channels in terminal and juxtaparanodal regions of neurons. *Nature* 1993;365:75–79.
34. Biervert C, Schroeder BC, Kubisch C, et al. A potassium channel mutation in neonatal human epilepsy. *Science* 1998;279:403–406.
35. Leppert M, Anderson VE, Quattlebaum T, et al. Benign familial neonatal convulsions linked to genetic markers on chromosome 20. *Nature* 1989;337:647–648.
36. Weckhuysen S, Mandelstam S, Suls A, et al. *KCNQ2* encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. *Ann Neurol* 2012;71:15–25.
37. Howell KB, McMahon JM, Carvill GL, et al. *SCN2A* encephalopathy: a major cause of epilepsy of infancy with migrating focal seizures. *Neurology* 2015;85:958–966.
38. Schwarz N, Hahn A, Bast T, et al. Mutations in the sodium channel gene *SCN2A* cause neonatal epilepsy with late-onset episodic ataxia. *J Neurol* 2016;263:334–343.
39. Larsen J, Carvill GL, Gardella E, et al. The phenotypic spectrum of *SCN8A* encephalopathy. *Neurology* 2015;84:480–489.
40. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform* 2013;14:178–192.

Practice Current: An interactive exchange on controversial topics

“When do you stop antiepileptic drugs in patients with genetic generalized epilepsies and in patients with focal epilepsies?”

Share your own best practices. Take the survey now!

Neurology® Clinical Practice <http://tinyurl.com/NCPPC>

Visit the *Neurology*® Resident & Fellow Website

Click on Residents & Fellows tab at Neurology.org.

Now offering:

- *Neurology*® Resident & Fellow Editorial team information
- “Search by subcategory” option
- E-pearl of the Week
- RSS Feeds
- Direct links to Continuum®, Career Planning, and AAN Resident & Fellow pages
- Recently published Resident & Fellow articles
- Podcast descriptions

Find *Neurology*® Residents & Fellows Section on Facebook: <http://tinyurl.com/o8ahsys>

Follow *Neurology*® on Twitter: <http://twitter.com/GreenJournal>