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BRIEF COMMUNICATION

Distinct epilepsy phenotypes and response to drugs in *KCNA1* gain- and loss-of function variants

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Abstract

A wide phenotypic spectrum of neurological diseases is associated with *KCNA1* (Kv1.1) variants. To investigate the molecular basis of such a heterogeneous clinical presentation and identify the possible correlation with in vitro phenotypes, we compared the functional consequences of three heterozygous de novo variants (p.P403S, p.P405L, and p.P405S) in Kv1.1 pore region found in four patients with severe developmental and epileptic encephalopathy (DEE), with those of a de novo variant in the voltage sensor (p.A261T) identified in two patients with mild, carbamazepine-responsive, focal epilepsy. Patch-clamp electrophysiology was used to investigate the functional properties of mutant Kv1.1 subunits, both expressed as homomers and heteromers with wild-type Kv1.1 subunits. *KCNA1* pore mutations markedly decreased (p. P405S) or fully suppressed (p. P403S, p. P405L) Kv1.1-mediated currents, exerting loss-of-function (LoF) effects. By contrast, channels carrying the p.A261T variant exhibited a hyperpolarizing shift of the activation process, consistent with a gain-of-function (GoF) effect. The present results unveil a novel correlation between in vitro phenotype (GoF vs LoF) and clinical course (mild vs severe) in *KCNA1*-related phenotypes. The excellent clinical response to carbamazepine observed in the patients carrying the A261T variant suggests an exquisite sensitivity of *KCNA1* GoF to sodium channel inhibition that should be further explored.

Francesco Miceli and Renzo Guerrini contributed equally to this work.

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KEYWORDS
developmental encephalopathies, epilepsy, gain-of-function variants, KCNA1, loss-of-function variants, potassium channels

1 | INTRODUCTION

A wide phenotypic spectrum of neurological diseases is associated with variants in the *KCNA1* gene encoding for neuronal Kv1.1 voltage-gated potassium channels. Although about 50% of all variants have been associated with isolated episodic ataxia type 1 (EA1),[1] the remaining 50% are associated with additional phenotypes, with limited overlapping, including EA1 with epilepsy, severe developmental and epileptic encephalopathy (DEE), myokymia, hyperthermia, and hypomagnesemia.[2,3]

Here, we describe the functional consequences of four pathogenic *KCNA1* variants, of which three, located within the highly conserved Pro-Val-Pro (PVP) pore motif and identified in patients with severe epilepsy or early onset DEE[2], resulted in a *loss-of-function* (LoF) effect, and one, located in the voltage sensor and identified in a patient with mild, drug-responsive focal epilepsy, caused *gain-of-function* (GoF) effects in vitro.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients 1–4 were reported previously in a clinical and genetic study describing severe, early onset epilepsy phenotypes associated with *KCNA1* genetic variants.[2] Patient 5 was referred for diagnostic workup and treatment of childhood-onset focal epilepsy and is described here for the first time. Clinical findings of the five patients are summarized in Table 1 and a detailed description of Patient 5 is presented in the "Results" section. This study was approved by the Paediatric Ethics Committees of the Tuscany Region, Italy.

2.2 | Genetic analysis

The methods for genetic testing in Patients 1–4 have been reported previously.[2] For Patient 5, genetic trio testing was performed using exome sequencing (ES) after informed consent. In brief, the Nextera Rapid Capture kit and a NextSeq 500 were used for the library preparation and the 2 × 100 bp paired-end sequencing.

2.3 | Mutagenesis and heterologous expression

Mutations were engineered in *KCNA1* human comlementary DNA (cDNA) cloned into pCMV6 (#RC211000; OriGene) by QuikChange site-directed mutagenesis (Agilent Technologies).[4,5] Channel subunits were expressed in Chinese Hamster Ovary (CHO) cells by transient transfection performed one day after seeding on glass coverslips using Lipofectamine 2000 (Invitrogen); a plasmid encoding for enhanced green fluorescent protein was used as a transfection marker; total cDNA in the transfection mixture was kept constant at 4 μg.

2.4 | Whole-cell electrophysiology

Currents were recorded at room temperature (20–22°C) 1–2 days after transfection, using an Axopatch 200B amplifier (Molecular Devices) and the whole-cell configuration of the patch-clamp technique, with glass micropipettes of 3–5 MΩ resistance.[4,5] The extracellular solution contained (in millimolar): 138 NaCl, 2 $CaCl_2$, 5.4 KCl, 1 $MgCl_2$, 10 glucose, and 10 HEPES, pH 7.4 with NaOH. The pipette (intracellular) solution contained (in millimolar): 140 KCl, 2 $MgCl_2$, 10 EGTA, 10 HEPES, and 5 Mg-ATP, pH 7.3–7.4 with KOH. The pCLAMP software (version 10.0.2) was used for data acquisition and analysis. Conductance-voltage curves were obtained and analyzed as described previously.[6]

2.5 | Statistics

Data were analyzed using SigmaPlot (Systat Software Inc.). Values are expressed as the mean ± standard error of the mean (SEM) of cells recorded in at least three independent experimental sessions. Statistically significant differences between the data were evaluated with the Student's *t* test ($p < .05$).

3 | RESULTS

3.1 | Clinical and genetic findings

Updated clinical and genetic findings in Patients 1–4 are summarized in Table 1.[3] The findings for Patient 5, in addition to Table 1, are also presented in detail below.

Patient 5. This young lady, now age 18, manifested since age 7 focal occipital seizures with blurred vision, followed by secondary generalization, appearing in clusters precipitated by fever, and accompanied by focal interictal and ictal electroencephalography (EEG) abnormalities in the right occipital lobe. Cognitive level was normal and brain magnetic resonance imaging (MRI) was unrevealing. Seizures responded to initial valproate treatment, which was started at age 8 but she relapsed at age 12, with similar characteristics, again, during a febrile episode soon after treatment was suspended. Valproate was reintroduced but seizures persisted and were video-EEG recorded as episodes of visual hallucinations of circles and blurred vision, lasting for about 30 s, accompanied by a sensation of fading hearing, at times followed by a secondary tonic-clonic phase.

At age 15, valproate was replaced by carbamazepine monotherapy, with immediate and complete seizure control during the following 3 years, with drug plasma levels at around 8 μg/mL during the following 3 years, apart from a single seizure cluster after reduced compliance. Whole exome sequencing identified a c.781G > A heterozygous variant in the *KCNA1* gene (NM_000217.3), confirmed to be de novo by Sanger sequencing and resulting in the missense substitution p.A261T. The variant was absent from the gnomAD population data set (gnomad.broadinstitute.org), not reported in the *HGMD* mutation database (portal.biobase-international.com/hgmd/pro/start.php) and predicted as pathogenic by several computational tools.[7] We classified the variant as likely pathogenic (PS2, PM1, PM2, PP2, PP3) according to the American College of Medical Genetics classification.[8] No other relevant variant was detected in the patient.

3.2 | Functional analysis

Kv1.1 subunits contain six transmembrane segments (S_1–S_6), with the S_1–S_4 region corresponding to the voltage sensing domain (VSD), and the S_5–S_6 segments and the intervening linker forming the ion selective pore.[9] The A261 residue is localized in the S_3 segment of the VSD, whereas the P403 and P405 residues fall within the highly conserved PXP motif localized at the bottom of the pore-forming S_6 segment (Figure 1A). CHO cells expressing homomeric Kv1.1 channels generate robust voltage-dependent K^+-selective currents that activate with fast kinetics with a threshold around −50 mV; when compared to Kv1.1, cells transfected with Kv1.1 A261T mutant cDNA displayed a 20 mV hyperpolarizing shift in steady-state activation gating (Figure 1B). By contrast, although Kv1.1 P403S- or Kv1.1 P405L-expressing cells failed to generate measurable currents, a marked (~70 mV) depolarizing shift in voltage-dependent activation gating and a significant reduction in current density was observed in CHO cells expressing Kv1.1 P405S (Figure 1B–D). To reproduce in vitro the genetic balance of the patients who carry one wild-type and one mutant allele, we transfected CHO cells with wild-type and mutant cDNAs in a 1:1 ratio. When compared to Kv1.1, cells expressing Kv1.1+Kv1.1 A261T subunits showed a hyperpolarizing shift in voltage-dependent activation, the extent of which was similar to that recorded in homomeric Kv1.1 A261T mutant channels (about -17 mV) (Figure 1D). By contrast, no currents could be recorded from Kv1.1 + Kv1.1 P403S- or Kv1.1 + Kv1.1 P405L-transfected cells, whereas activation of currents from Kv1.1+Kv1.1 P405S-transfected cells was positively shifted by 45 mV, displaying therefore a $V_{1/2}$ intermediate between that of homomeric Kv1.1 and Kv1.1 P405S channels (Figure 1C, D). Table S1 summarizes the detailed biophysical properties of the currents carried by the described experimental groups.

4 | DISCUSSION

In vitro assessment of the functional consequences of rare variants in ion channel genes, can reveal pathogenic mechanisms and genotype-phenotype correlations, and is critical for diagnostic evaluation, prognostic predictions, and, possibly, a more tailored therapeutic management of patients with a wide spectrum of neurological disorders. Mutations in the *KCNA1* gene are among the most frequent cause of EA1, which is associated with epilepsy in some patients.[3] Additional, rare phenotypes associated with *KCNA1* mutations include paroxysmal dyskinesias, severe developmental and epileptic encephalopathies, and hypomagnesemia.[2,3]

Although the pathogenetic mechanisms for such a wide variety of symptoms for *KCNA1*-associated phenotypes is only partly understood, in vitro functional analysis has consistently revealed that, regardless of the associated phenotype, incorporation of mutant subunits decreases channel function, suggesting a LoF pathogenetic mechanism. However, genotype-phenotype correlations have been quite elusive due to both the rarity of variant occurrence (only about 50 *KCNA1* variants have been described until now; HGMD 2021.2) and the lack of correlations between in vitro functional consequences and disease severity.

In this study, the functional consequence of a de novo *KCNA1* variant (p. A261T) found in a patient with mild, carbamazepine-sensitive, childhood-onset focal epilepsy without ataxia was investigated. The same variant also occurred in a young girl with carbamazepine-sensitive focal epilepsy, accompanied by rare ataxic and myokymic episodes.[10] Our findings indicate that channels incorporating mutant subunits, both in homomeric and heteromeric configuration with wild-type subunits, were fully functional and displayed a marked increase in their voltage-sensitivity, consistent

TABLE 1 Clinical description of patients with *KCNA1* variants functionally characterized

	Genetic findings					Clinical findings		
Patient	Nucleotide change (NM_000217)	Amino acid substitution	gnomAD frequency	Inheritance	Functional domain	Phenotype	Age at seizure onset	Age at last \follow-up
1/M	c.1213C > T	p. Pro405Ser	—	de novo	S6 – PVP motif	Epileptic encephalopathy with severe cognitive impairment	3 m	5 y
2/F	c.1214C > T	p. Pro405Leu	—	de novo	S6 – PVP motif	Epileptic encephalopathy with severe cognitive impairment	18 d	7 y 6 m
3/M (identical twin brother of Patient 4)	c.1207C > T	p. Pro403Ser	—	de novo	S6 – PVP motif	Generalized epilepsy, moderate cognitive impairment & myokymia	6 m	20 y
4/M (identical twin brother of Patient 3)	c.1207C > T	p. Pro403Ser	—	de novo	S6 – PVP motif	Epilepsy, severe cognitive impairment & myokymia	4 m	20 y
5/F	c.781G > A	p. Ala261Thr	—	de novo	S3	Focal epilepsy responsive to carbamazepine	7 y	18 y

Abbreviations: CBZ, carbamazepine; Clob, clobazam; d, days; F, female; GVG, vigabatrin; LTG, lamotrigine; M, male; m, months; NA, not available; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; VPA valproic acid; y, years; ZSM, zonisamide.

Seizure types/treatments used	EEG findings	Brain MRI	Additional clinical features	Described in:	Additional references
3 m: back arching spells; 5 m: brief staring spell that was followed by flaccidity of the arms and legs; 18 m: prolonged episodes (>30 min) with unresponsiveness and leftward eye deviation, with or without clonic movements; 5 y: 3 to 4 prolonged seizures per year, often precipitated by fever; /PB, Clob, CBZ, ZSM and VPA, all ineffective	3 m: generalized slowing;18 m: subclinical seizure with left parietal onset, high-amplitude, multifocal epileptiform abnormalities and generalised slowing	3 m: symmetric subdural fluid collections and prominent subarachnoid spaces; 5 y: normal	Developmental delay, mild to moderate axial and appendicular hypotonia, increased insertional activity consistent with irritable motor nerves at electromyography but without classic myokymic discharges.	Rogers et al., 2018	—
18 d: generalized clonic; 2 m: generalized tonic-clonic seizures; 4 y: focal onset impaired awareness seizures; often precipitated by fever; PB, CBZ, LEV, and PHT all ineffective or very short benefit	18 d: severe epileptiform discharges on both hemispheres; 4 y: multifocal abnormalities, with left temporal predominance; 7 y: severe bilateral independent epileptiform discharges, with status epilepticus during sleep (ESES) responsive to ACTH	normal	Developmental delay, autism spectrum disorder, moderate-severe intellectual disability, distal tremor and impaired coordination of cerebellar origin	Parrini et al., 2017	Russo et al., 2020
6 m: generalized tonic-clonic seizures; LTG partially effective, multiple additional medications not specified, all ineffective	NA	6 m: normal	Developmental delay, ataxia, intellectual disability severe recurrent headaches	Rogers et al., 2018	—
Generalized tonic-clonic and absence seizures; LTG, GVG, OXC, VPA, PHT, and Clob, all ineffective	NA	2 y: high signal intensities in the globus pallidus, slightly delayed myelination	Developmental delay, ataxia, myokymia, moderate intellectual disability. Between 8–12 y, language loss and walk only with support	Rogers et al., 2018	—
Focal occipital seizures with blurred vision, complex hallucinations and, at times, fading hearing; secondarily generalized tonic-clonic seizures Seizure precipitation during fever; VPA transiently effective, CBZ effective	Bilateral, right predominant, occipital spikes	8 y: normal	None	This paper	Yuan et al., 2020

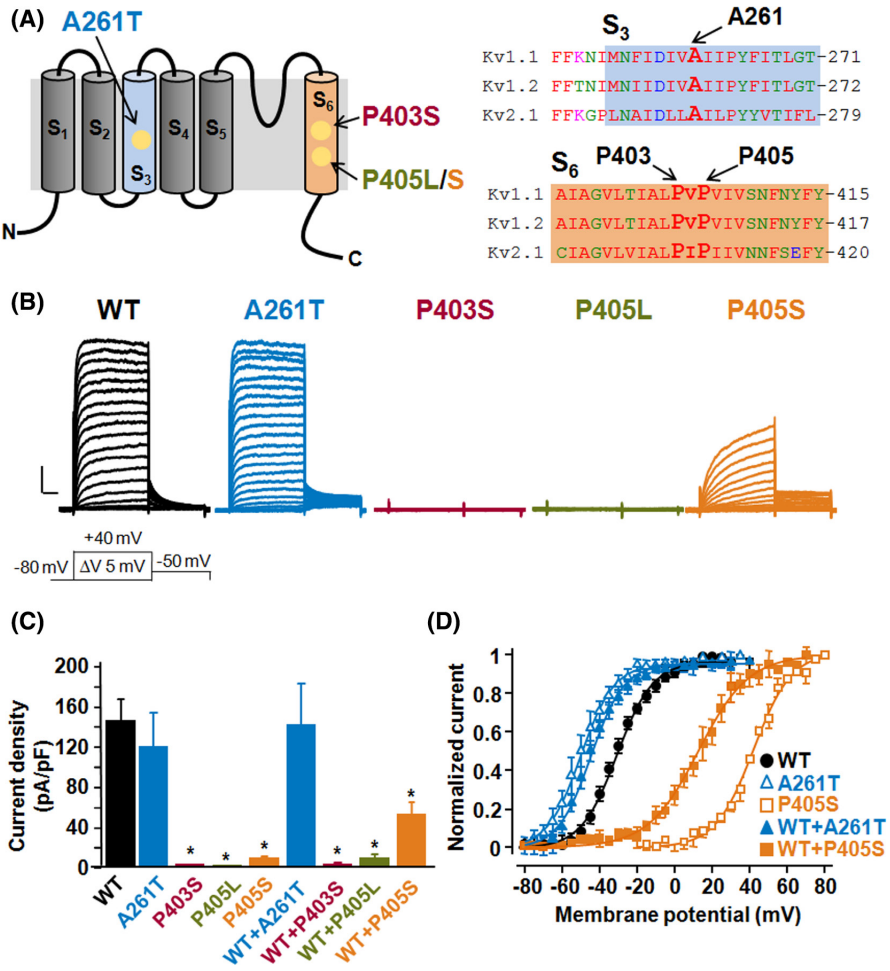

FIGURE 1 Location and functional characterization of Kv1.1 mutants. (A) Cartoon depicting the six transmembrane segments arrangement of a single Kv1.1 subunit (left panel) and sequence alignments of the S_3 (top right panel) and S_6 (lower right panel) region of Kv1.1, Kv1.2, and Kv2.1 subunits. (B) Macroscopic currents recorded from CHO cells transfected with plasmids encoding for wild-type (WT) or the indicated mutant Kv1.1 subunits, in response to the voltage protocol shown below the Kv1.1 traces. Current scale, 500 pA; time scale, 40 ms. (C) Current density (expressed as pA/pF) at +20 mV from CHO cells expressing each of the indicated experimental group. Asterisks (*) indicate values significantly different from each respective control ($p < .05$). (D) Conductance/voltage curves for each of the indicated groups. Continuous lines are Boltzmann fits to the experimental data. Each data point is the mean ± SEM. of 13–21 cells recorded in at least three separate experimental sessions.

with a GoF pathogenetic mechanism. Notably, since the first functional description of a disease-causing variant over 25 years ago,[1] this is the first variant in *KCNA1* in which GoF effects are demonstrated. By contrast, the three variants affecting two critical residues in the PVP pore motif (p. P403S, p. P405L, and p. P405S) occurring in patients with severe DEE,[2,11,12] all caused strong LoF in vitro changes. Notably, within the same PVP region, another *KCNA1* variant (p. V404I) causing a dominantly transmitted, familial EA1 phenotype and responsible for milder in vitro LoF effects has been described.[13] Altogether these data are suggestive of novel genotype-phenotype correlations for the *KCNA1* gene, with GoF effects associated with drug-sensitive epilepsy with no neurodevelopmental impairment, and strong LoF effects in the PVP region responsible for a severe phenotype of pharmacoresistant DEE with intellectual disability.

It is intriguing that a paralogous variant affecting the first proline of the PVP domain in *KCNA2*-encoded Kv1.2 subunits (p. P405L), recurrently found in patients with DEE and mild-moderate intellectual disability, also led to strong dominant-negative LoF effects.[14] As shown herein for *KCNA1*, both LoF and GoF variants are associated with distinct clinical phenotypes within the *KCNA2*-related diseases spectrum,[15] raising the possibility that the in vitro electrophysiological phenotype might help in predicting pathogenetic mechanisms and treatment possibilities. 4-Aminopyridine has recently been shown to be a promising treatment for patients with GoF *KCNA2*-encephalopathy[16]; it remains to be investigated whether this drug might represent a potential therapeutic option also for patients with epilepsy due to GoF variants in *KCNA1*.

Structural data from highly homologous Kv1.2 subunits may allow speculation about the molecular basis for the LoF and GoF effects herein described. The conserved PVP sequence represents the pore activation gate.[9] In the closed channel, the four subunits come in close contact with each other at the PVP region to impede ion passage; during activation, the outward displacement of the VSD, via the S_4–S_5 linker, disrupts PVP intersubunit interactions and leads to pore opening.[17] Therefore, the PVP prolines provide the S_6 region with the degree of mobility needed for channel gating[18]; substitution of these prolines with nonpolar (L) or polar (S) residues might reduce S_6 flexibility, thereby impeding the structural transition linking VSD movement to pore opening. On the other hand, the Kv1.2 residue corresponding to A261 (A262) in S_3 is in a narrow (10 Å) hydrophobic region in the VSD where the transmembrane electric field is focused[19]; introduction of a polar T residue at this position might affect such "focused field," facilitating charge transfer during VDS displacement and causing GoF consequences.

In vivo, Kv1.1 subunits are abundantly expressed in neurons, where they regulate neuronal excitability; axonal expression of these channels repolarizes the action potential and regulates firing frequency, whereas presynaptic Kv1.1-containing channels blunt neurotransmitter release. Given their inhibitory role on neuronal excitability, the herein found GoF effects of an epilepsy-causing *KCNA1* variant may seem paradoxical. However, GoF variants causing neuronal hyperexcitability have been described in several K^+ channel–encoding genes,[20] and it has been suggested that faster action potential repolarization may lead to sodium channels repriming,[21] thereby increasing firing frequency and synchronization, hallmarks of neuronal hyperexcitability in the epileptic tissue. The marked anticonvulsant efficacy of the sodium-channel blocker carbamazepine observed in our patient and in Patient 1 of Yuan et al.,[10] who also carried the same p. A261T *KCNA1* variant, but not in those with loss-of-function mutations, appears to support such a hypothesis.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

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