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BRIEF COMMUNICATION

A novel *KCNC1* gain-of-function variant causing developmental and epileptic encephalopathy: "Precision medicine" approach with fluoxetine

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Abstract

Variable phenotypes, including developmental encephalopathy with (DEE) or without seizures and myoclonic epilepsy and ataxia due to potassium channel mutation, are caused by pathogenetic variants in *KCNC1*, encoding for Kv3.1 channel subunits. In vitro, channels carrying most *KCNC1* pathogenic variants display loss-of-function features. Here, we describe a child affected by DEE with fever-triggered seizures, caused by a novel de novo heterozygous missense *KCNC1* variant (c.1273G>A; V425M). Patch-clamp recordings in transiently transfected CHO cells revealed that, compared to wild-type, Kv3.1 V425M currents (1) were larger, with membrane potentials between −40 and +40 mV; (2) displayed a hyperpolarizing shift in activation gating; (3) failed to inactivate; and (4) had slower activation and deactivation kinetics, consistent with a mixed functional pattern with prevalent gain-of-function effects. Exposure to the antidepressant drug fluoxetine inhibited currents expressed by both wild-type and mutant Kv3.1 channels. Treatment of the proband with fluoxetine led to a rapid and prolonged clinical amelioration, with the disappearance of seizures and an improvement in

Paolo Ambrosino and Francesca Ragona contributed equally to this study.

balance, gross motor skills, and oculomotor coordination. These results suggest that drug repurposing based on the specific genetic defect may provide an effective personalized treatment for *KCNC1*-related DEEs.

KEYWORDS

drug repurposing, epilepsy, fluoxetine, *KCNC1*, next generation sequencing, precision medicine

1 | INTRODUCTION

Developmental and epileptic encephalopathies (DEEs) are often caused by variants in neuronally expressed genes, such as those encoding for voltage-gated K^+ channels (Kv channels). Among these, *KCNC1* encodes for Kv3.1 subunits belonging to the Shaw family, which includes Kv3.1-Kv3.4 subunits forming functional homo- or heterotetrameric channels. Each Kv3 subunit shows the typical arrangement with six transmembrane segments (from S_1 to S_6), including the voltage sensor domain (from S_1 to S_4) and the pore domain (PD; encompassing the S_5, S_6 and the intervening linker).[1] Kv3.1 channels are activated by strong membrane depolarizations and display very fast activation and deactivation kinetics. They are mainly expressed in the brain, being particularly abundant in parvalbumin-positive γ-aminobutyric acidergic interneurons that fire long trains of action potentials at high frequency (fast-spiking neurons).

To date, only 11 pathogenetic variants of *KCNC1* have been reported, mostly associated with severe neurological phenotypes, including intellectual disability, epilepsy, and/or ataxia. Among them, eight show loss-of-function (LoF), whereas three showed gain-of-function (GoF) consequences when studied in vitro. Among LoF variants, the R320H, affecting the fourth of the highly conserved arginine residues of the S_4 transmembrane segment, is the most recurrent variant, responsible for progressive myoclonus epilepsy, currently defined as "myoclonus epilepsy and ataxia due to potassium channel mutation"[2,3]; in the same segment, a further variant (R317H) was identified in a patient with myoclonic epilepsy, ataxia, and distinct radiological features.[4] Other missense LoF variants have been identified in patients with isolated nonprogressive myoclonus (C208Y; S_1), intellectual disability (T399M; S_5–S_6 linker), or epilepsy with myoclonic, absence, and generalized tonic–clonic seizures, ataxia, and developmental delay (A421V, three patients; S_6)[5]; by contrast, the A513V variant, found in a patient with epilepsy of infancy with focal migrating seizures,[6] renders Kv3.1 channels insensitive to regulation by phosphorylation at S503, a nearby regulatory site in the C-terminus.[7]

Two additional nonsense LoF variants have been identified, one (R339X) in a proband with intellectual disability without epilepsy,[8] the other (Q492X) in three patients (including two familiar cases) with developmental encephalopathy.[6] More recently, three de novo heterozygous *KCNC1* GoF variants (M430I, V432M, and V434L) were described in three patients with developmental delay and hypotonia, without epilepsy or myoclonus.[9]

In the present study, we report a patient with DEE carrying a previously unreported de novo missense *KCNC1* variant (V425M) characterized by an in vitro mixed functional pattern with prevalent GoF effects, and we show that fluoxetine counteracted the mutation-induced functional defects in vitro, resulting in a significant and long-lasting clinical improvement when administered to the proband.

2 | MATERIALS AND METHODS

Materials and Methods are reported as Supplementary material.

3 | RESULTS

3.1 | Case description and genetic analysis

The proband is an 8-year-old girl without familial history of epilepsy and/or febrile seizures. She was born at term, after a normal pregnancy; APGAR score at birth was normal. Perinatal period and the first year of life were uneventful. The child presented mild motor delay; she achieved trunk control at 10 months and started to walk independently at 17 months. During the first year of life, language development was normal, and she spoke her first words at 12 months.

At 16 months of age, she presented with episodes of sudden startle while falling asleep, followed by rhythmic limb jerks and awakening; these episodes were not brought to medical attention. The patient came to our attention at 26 months for seizures characterized by (1) tonic–clonic movements during fever (both isolated and in cluster) and (2) episodes of rhythmic limb jerks during sleep. Her neurological examination

revealed hypotonia, mild ataxia, and gait apraxia. General developmental quotient assessed with the Griffiths Scale was 80, with particular impairment in fine motor skills, manual dexterity, and visual perception skills; expressive language was characterized by a few single words. Interictal electroencephalography (EEG) documented poor sleep organization with high-amplitude paroxysms of diffuse sharp waves (Figure 1A). At 3 years, video-EEG recording documented seizures characterized by clonic limb jerks occurring during sleep (Figure 1B, Video S1). Other recordings in

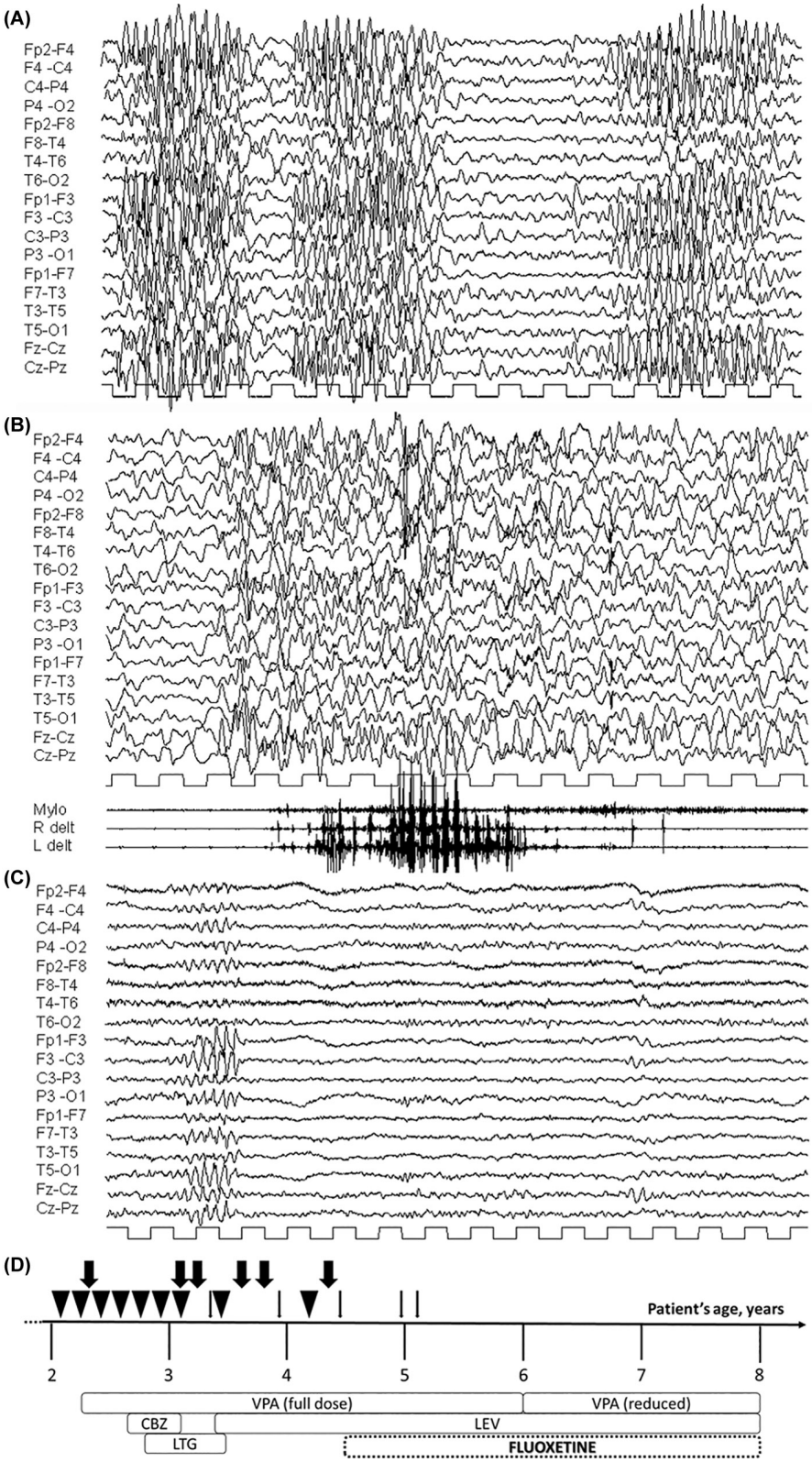

FIGURE 1 Patient's electroencephalographic (EEG) traces and pharmacological treatment. (A) EEG during sleep registered at the age of 2 years shows high-amplitude paroxysm of diffuse sharp waves. (B) Seizure recorded during sleep at the age 3 years, characterized by diffuse EEG rhythmic activity composed of sharp waves, associated with bilateral clonic movements. (C) Compared to previous examinations performed in the absence of fluoxetine, EEG at the age of 8 years shows a better overall organization, with shorter sharp wave paroxysm prevalent on the left frontocentral derivations. (D) Treatment with multiple antiseizure medications at full dosage did not result in satisfactory seizure control. At the age of 4.5 years, the introduction of fluoxetine resulted in the disappearance of seizures. Thin arrows represent single febrile seizures; large arrows represent clusters of febrile seizures; arrowheads represent daily focal seizures without fever. CBZ, carbamazepine; LEV, levetiracetam; LTG, lamotrigine; VPA, valproic acid.

the following months documented more prolonged seizures with similar features (Videos S2 and S3). Brain magnetic resonance imaging, cerebrospinal fluid glucose, karyotype, and *PCDH19* gene analysis were unrevealing. A gene panel targeted for epilepsy revealed the novel heterozygous missense variant c.1273G>A in the *KCNC1* gene, causing the p.V425M substitution in Kv3.1. Segregation analysis showed that this variant was absent in her parents, resulting de novo in the proband.

3.2 | Functional properties of Kv3.1 V425M mutant channels

The Kv3.1 V425M variant falls in the PD (Figure 2A), affecting an S_6 residue highly conserved among Kv subunits across species (Figure 2B). Patch-clamp recordings from transiently transfected CHO cells revealed that Kv3.1 channels carry robust (pA/pF at +60 mV was 663.9 ± 54.1; $n = 25$), rapidly activating outwardly rectifying K^+ currents with a threshold of activation > −30 mV, a $V_{½}$ of activation of −13.2 ± 1.3 mV, and a slope of the conductance/voltage (G/V) curve (k) of 14.4 ± 1.0 mV/e-fold (Figure 2C–E).[10] Kv3.1 currents inactivated at membrane potentials of ≥0 mV, showing fast kinetics (τ_{inact} at +60 mV was 2.9 ± .2 ms; $n = 26$); the extent of inactivation was the highest (~20%) at +60 mV (Figure 2C,G). Kv3.1 currents also showed fast and voltage-dependent deactivation kinetics (Figure 2F).

When compared to Kv3.1 channels, Kv3.1 V425M currents showed a ~30-mV hyperpolarizing shift in their activation voltage-dependence ($V_{½} = -46.7 \pm .7$ mV; $n = 29$; $p < .05$), with no concomitant change in k (11.5 ± .5 mV/e-fold; $p > .05$; Figure 2C,D). Moreover, Kv3.1 V425M currents displayed slower current activation (Figure 2E) and deactivation (Figure 2F) kinetics, and absence of inactivation (Figure 2C,G). No significant change occurred in maximal current densities (pA/pF at +60 mV were 670.7 ± 39.1; $n = 29$; $p > .05$ vs. wild-type channels; Figure 2C,D), although, given the negative shift in activation gating, these were significantly larger at membrane potentials between −50 and +40 mV (Figure 2D).

Coexpression of wild-type and mutant Kv3.1 channels, reproducing the genetic balance of the proband carrying the V425M variant in heterozygosis, produced qualitatively similar, but quantitatively smaller effects on voltage-dependence of activation ($V_{½}$ was −23.0 ± 2.1 mV; $n = 14$; $p < .05$ vs. respective homomers; Figure 2C,D), activation (Figure 2E) and deactivation (Figure 2F) kinetics, and inactivation (Figure 2G).

Overall, whereas the hyperpolarizing shift in activation, the lack of inactivation, and the slower deactivation kinetics predicted a GoF effect triggered by the V425M variant, the slower current activation kinetics were instead consistent with LoF effects. Thus, this variant displayed a combination of both GoF and LoF effects, although GoF effects appeared to be dominant, given the strong hyperpolarization shift in activation gating.

3.3 | Inhibition of Kv3.1 channels by fluoxetine

To counteract the mutation-induced GoF changes, we tested in vitro the effect of fluoxetine, a known Kv3.1 blocker,[11,12] on Kv3.1 channels carrying wild-type and mutant subunits (Figure 2H). Fluoxetine dose-dependently similarly blocked the currents carried by homomeric Kv3.1 and Kv3.1 V425M channels, as well as by heteromeric Kv3.1 + Kv3.1 V425M channels, being slightly more potent when mutant subunits were present (Figure 2I). These results suggested that fluoxetine largely reverted the functional changes triggered by the V425M mutation.

3.4 | "Precision" approach with fluoxetine

During follow-up, the proband was resistant to all antiseizure medications (ASMs) administered at a full dosage (Figure 1D). Based on the previously described in vitro data, at the age of 4.5 years the patient started treatment with a low dose of fluoxetine (6 mg/day), with a rapid and dramatic reduction of seizures. At 5 years of age, the patient achieved seizure freedom; the fluoxetine plasma concentration was 171.3 ng/mL. At 6 years, the dosage of valproic acid was reduced from 40 to 10 mg/kg/day, without recurrence of episodes. No seizures were recorded during serial EEGs performed at follow-up (Figure 1C). During fluoxetine treatment, the patient also reported an improvement in motor abilities and language development. At last follow-up (8 years), she was seizure-free, with a significant improvement in balance, gross motor skills, and oculomotor coordination. General development, assessed with the Griffiths Scale, was 53, confirming a modest psychomotor delay.

4 | DISCUSSION

We report the novel V425M de novo heterozygous missense Kv3.1 pathogenetic variant, identified in a patient with drug-resistant seizures triggered by fever and mild developmental delay. When compared to wild-type,

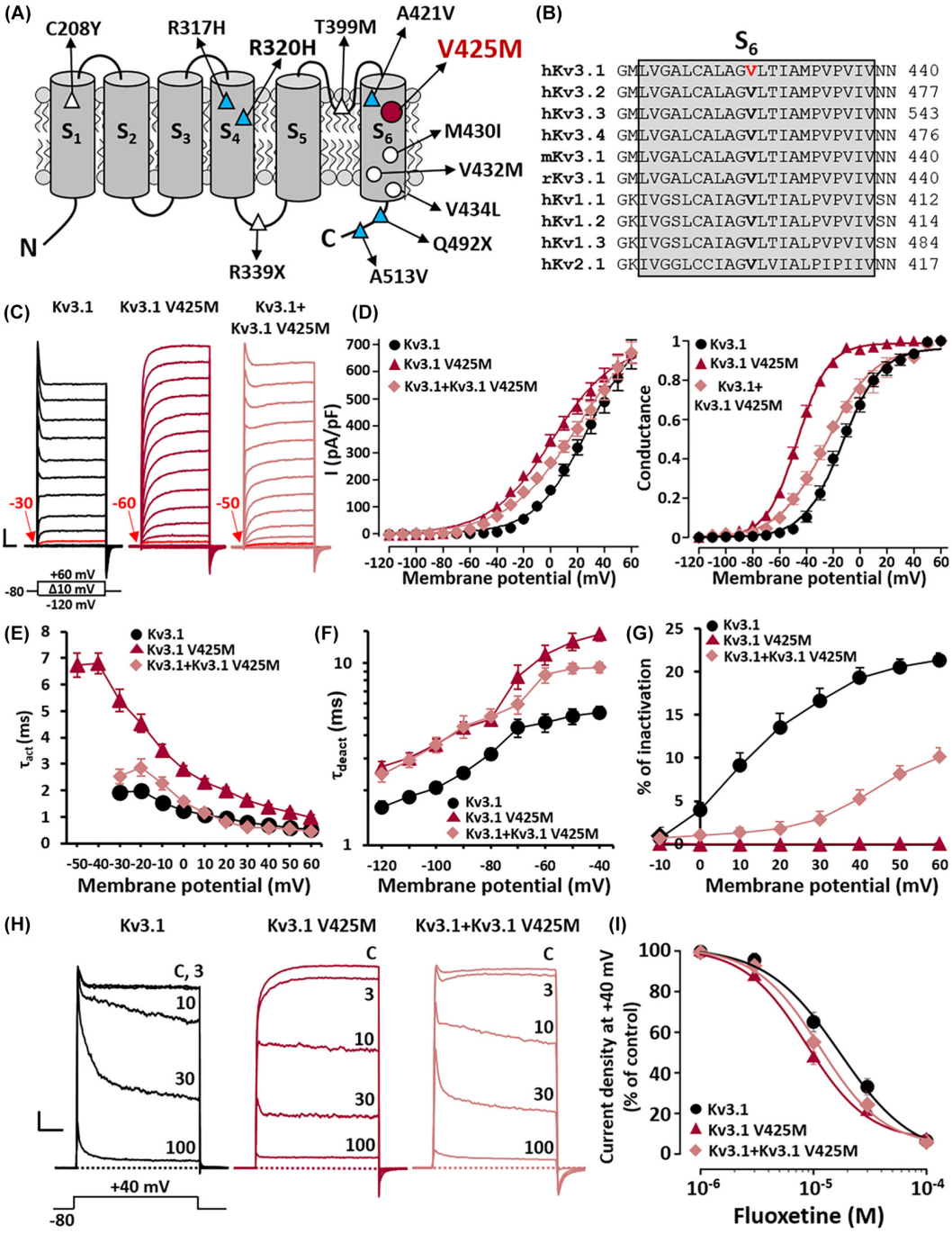

FIGURE 2 Functional properties of Kv3.1 channels carrying the V425M variant. (A) Topological structure of a Kv3.1 subunit. Circles indicate gain-of-function variants, triangles loss-of-function variants. Variants occurring in patients with or without epilepsy are indicated in light blue or white, respectively; the variant herein investigated is indicated in dark red. (B) Partial alignment among channels of the Kv3 family and other K^+ channels; the red V indicates herein investigated mutated residue. (C) Representative current traces measured upon exposure to the voltage protocol shown below the first group of traces, in CHO cells transiently expressing the indicated channels. The arrows and numbers in red indicate the position and value of the voltage threshold for current activation. Current scale: 1 nA; time scale: 20 ms. (D) Average current density (left panel) and conductance (right panel) measured in experiments like those shown in panel C performed in CHO cells transiently expressing the indicated channels. (E–G) Average of faster activation kinetics (E), deactivation kinetics (F), and percentage of current inactivation (G) measured in CHO cells transiently expressing the indicated channels. (H, I). Representative currents traces (H) and percentage of current inhibited (I) measured in CHO cells transiently expressing the indicated channels, upon application of the indicated voltage protocol, in control solution (C) or upon exposure to 3, 10, 30, or 100 $\mu mol \cdot L^{-1}$ fluoxetine, as indicated. The half-maximal inhibitory concentrations were $17.0 \pm 2.7\ \mu mol \cdot L^{-1}$, $8.7 \pm .8\ \mu mol \cdot L^{-1}$, and $11.4 \pm .1\ \mu mol \cdot L^{-1}$ in KCNC1, KCNC1 V425M, and KCNC1 + KCNC1 + KCNC1 V425M channels, respectively ($p < .05$ vs. KCNC1). Current scale: 1 nA; time scale: 20 ms.

mutant channels revealed a significant hyperpolarizing shift in voltage-dependence of activation, slower activation and deactivation kinetics, and absence of inactivation, functional features largely compatible with a GoF in vitro phenotype. Qualitatively similar biophysical consequences on channel function in vitro have been recently described in association with three *KCNC1* heterozygous variants affecting S_6 residues different from that herein described, found in patients with isolated developmental delay but without epilepsy. Cryogenic electron microscopy structural data from human Kv3.1 channels[13] have recently revealed that the side chain from the highly conserved V425 residue projects toward the pore cavity (Figure 2B), in a region where other residues (such as T397, T400, and Y403) are located and play a critical role in the slow inactivation process (Figure S1A).[14] In silico analysis revealed that the substitution of a smaller, aliphatic, and nonionizing V with a bulkier, thioether, and ionizing M residue at position 425 reduces the distance between the 425 and 397 residues (Figure S1B), possibly prompting the herein-described variant-dependent functional changes.

To identify repurposed drugs able to revert the in vitro GoF changes triggered by the V425M variant, fluoxetine, a known open channel blocker of Kv3.1 channels,[12] was also found to produce a dose-dependent block of the currents carried by mutant channels, showing a slight increase in potency when compared to wild-type currents. Based on these in vitro data and considering the scarce response to ASMs, fluoxetine was administered to the proband, leading to a complete disappearance of seizures during a prolonged follow-up. Notably, at steady-state, the plasma level of fluoxetine was within the suggested antidepressant range during chronic treatment (120–500 ng/mL, corresponding to a total drug concentration of .6–2 $\mu mol \cdot L^{-1}$). Considering that only 5% of the drug is not protein bound, but that, being very lipophilic, fluoxetine can reach elevated concentrations in the brain (the plasma/brain ratio is about .1),[15] and that the major metabolite norfluoxetine blocks Kv3.1 current with potency even higher when compared to the parent compound,[11] it seems reasonable to assume that significant target occupancy occurred with fluoxetine at the level of brain Kv3.1 channels, and that this action contributes to the clinical improvement observed in vivo in the proband.

The observation that both GoF and LoF effects in K^+ channels cause epileptic phenotypes is not novel, although how distinct pathogenetic mechanisms lead to apparently similar phenotypes is currently unknown.[16] Kv3.1 channels are mainly expressed in fast-spiking inhibitory neurons, where they contribute to action potential repolarization without affecting the threshold for action potential generation, thereby allowing high-frequency firing.[1,17] Thus, we may speculate that the Kv3.1 V425M GoF variant, similarly to other GoF pore variants,[9] may hyperpolarize the resting membrane potential and hamper fast-spiking activity of these neurons in vivo, thus increasing the excitation/inhibition balance that triggers epileptic activity.

Our data also allow formulation of some important genotype–phenotype correlations regarding the role of fever in seizure occurrence in *KCNC1*-related disorders. Patients carrying the R320H LoF variant typically experience a transient clinical improvement during fever, an effect interpreted as a consequence of the temperature-dependent increase in channel function caused by the combinatorial effects of the hyperpolarizing shift in the activation voltage-dependence and the reduced inactivation; both effects would at least partially restore wild-type functional behavior.[18] Instead, variant-dependent GoF changes would be aggravated by increased temperatures, possibly explaining the worsening of seizures associated with increased body temperature as observed in the proband here described.

5 | CONCLUSIONS

Widespread use of next generation sequencing techniques has markedly improved knowledge of the etiology and pathogenetic mechanisms of DEEs. Treatment with repurposed drugs selected on the basis of the patient's specific genetic defect and on its functional properties when expressed in vitro represents one of the possible strategies for a "precision medicine" approach. We herein show that a novel pathogenic variant in *KCNC1* encoding for Kv3.1 voltage-gated potassium channel subunits caused fluoxetine-sensitive GoF effect in vitro, and that fluoxetine treatment of the proband led to a marked seizure suppression and to a significant behavioral improvement, thus providing a successful example of such a drug repurposing strategy. Notably, large-scale studies are needed to establish whether fluoxetine treatment might provide similar clinical benefits also in patients with other pathogenic GoF variants in *KCNC1* or in other potassium channels.[19]

AUTHOR CONTRIBUTIONS

Paolo Ambrosino, Ilaria Mosca, Ilaria Rivolta, and Maria Virginia Soldovieri collected experimental data. Francesca Ragona, Chiara Vannicola, Roberta Solazzi, Elena Freri, and Tiziana Granata collected clinical data. Laura

Canafoglia collected EEG data. Giuliana Messina, Barbara Castellotti, and Cinzia Gellera collected next generation sequencing genetic data. Jacopo Cosimo DiFrancesco, Maria Virginia Soldovieri, and Maurizio Taglialatela contributed to conception and design of the study, and wrote the manuscript. All authors contributed to manuscript revision, and read and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. 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

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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