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Gabapentin treatment in a patient with KCNQ2 developmental epileptic encephalopathy

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ABSTRACT

De novo variants in KCNQ2 encoding for Kv7.2 voltage-dependent neuronal potassium (K^+) channel subunits are associated with developmental epileptic encephalopathy (DEE). We herein describe the clinical and electroencephalographic (EEG) features of a child with early-onset DEE caused by the novel *KCNQ2* p.G310S variant. *In vitro* experiments demonstrated that the mutation induces loss-of-function effects on the currents produced by channels incorporating mutant subunits; these effects were counteracted by the selective Kv7 opener retigabine and by gabapentin, a recently described Kv7 activator. Given these data, the patient started treatment with gabapentin, showing a rapid and sustained clinical and EEG improvement over the following months. Overall, these results suggest that gabapentin can be regarded as a precision therapy for DEEs due to *KCNQ2* loss-of-function mutations.

1. INTRODUCTION

De novo variants (DNVs) in KCNQ2 are major causes for early-onset developmental epileptic encephalopathy (DEE), with characteristic drug resistance and poor short-term prognosis in most patients [1]. *KCNQ2* encodes for Kv7.2 voltage-dependent neuronal potassium (K^+) channel subunits which, upon heteromerization with Kv7.3 subunits encoded by the highly homologous *KCNQ3* gene, represent the primary molecular correlate of a current known as the M-current (I_{KM}) which exerts an inhibitory control over neuronal excitability [2,3]. The largest fraction of *KCNQ2* DNVs causes loss-of-function (LoF) effects on channels incorporating mutant Kv7.2 subunits, and the extent of mutation-induced LoF may correlate with clinical disease severity [4–6]. Therefore, specific Kv7 activators could represent a rational treatment for DEE-affected patients carrying LoF KCNQ2 DNVs [7]. However, two Kv7 openers, retigabine (RTG) and its analogue flupirtine [8], are no longer commercially available. Recent evidence revealed that gabapentin (GBP), a widely used anticonvulsant and analgesic drug [9], acts as a potent activator of Kv7 currents [10]. In fact, although the therapeutic actions of gabapentinoids such as GBP and pregabalin have been largely, but not exclusively, attributed to their ability to bind to $\alpha_2\delta$ subunits of voltage-gated calcium channels and reduce neuronal calcium currents [11], GBP (but not pregabalin) has been shown to activate homomeric Kv7.3 and heteromeric Kv7.2/3 voltage-gated potassium

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channels. Noteworthy, GBP-induced Kv7 current activation was critically dependent on the presence of the same conserved tryptophan residue in the 5th transmembrane segment (S_5) required for retigabine's actions [10]. Herein we describe the clinical and EEG features of a child with early-onset DEE caused by the novel *KCNQ2* p.G310S variant. *In vitro* experiments demonstrated that the mutation induces LoF effects on the currents produced by channels incorporating mutant subunits, and that these effects were counteracted by the selective Kv7 opener retigabine as well as by gabapentin. Finally, we also report the child response to GBP used for the first time as a precision therapy in a DEE-affected child carrying a novel LoF DNV in KCNQ2.

2. METHODS

2.1. Genetic analysis

Experimental protocols were approved by the Institutional Review Boards of the Fondazione IRCCS Istituto Neurologico Carlo Besta. Genomic DNA was prepared from peripheral blood lymphocytes using standard procedures by liquid handing Freedom Evo (Tecan) using the NucleoSpin Blood L Vacum kit (Macherey-Nagel). Written informed consent was requested for the patient and her parents.

To identify the possible pathogenic mutations related to the patient's phenotype, we designed an NGS gene panel targeted for epileptic encephalopathies [12,13], using a Nextera Rapid Capture Custom Enrichment Kit (Illumina). The average NGS coverage at 20X for this patient was 99%. The resulting sequences were aligned to the reference genome (GRCh37 / hg19) using the MiSeq software. The data analysis was performed with the following softwares: Illumina MiSeq Reporter vs 2.4.60, Illumina Variant Studio vs 2.2, and Qiagen CLC Genomics Workbench vs 7.0. Variants with MAF > 1% reported in the dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genome (browser.1000genomes.org), EVS database (evs.gs.washington. Edu), the Genome Aggregation database (https://gnomad.broadinstitute.org/) and Varsome (https://varsome.com/) were considered benign variants and excluded from the report. The segregation of variants has been carried with the Sanger direct sequencing using an Applied Biosystems 3130 XL sequencer.

2.2. mRNA splicing analysis

Validation of the effects prompted by the c.928 G > A mutation on KCNQ2 mRNAs splicing was performed as previously described for the KCNQ2 c.928-1G > A substitution [14]. RNA was extracted from Epstein-Barr Virus-immortalized lymphoblastoid cells of the proband and her parents with the RNeasy minikit (Qiagen, Courtaboeuf, France). Retrotranscription was performed using the Expand RT kit (Invitrogen, Saint Aubin, France). PCR was performed with primers in exon 5 (forward: 5'-TACGCGGATGCACTCTGG-3') and exon 8 (reverse: 5'-GTGGAGTGCAGGTCTGTGC-3'); PCR products were subsequently sequenced. cDNA amplicons were separated on a gel electrophoresis and analyzed using a Labchip GX (Perkin Elmer).

2.3. Mutagenesis, heterologous expression, and functional analysis in CHO cells

The c.928 A > G substitution was engineered by Quick-change mutagenesis (Agilent Technologies) in pcDNA3.1-Kv7.2 or EGFP-Kv7.2-HA plasmids as previously reported [14]. Protocols for Chinese hamster ovary (CHO) cells growth, transfection procedures and patch-clamp experiments in CHO cells and neurons were reported previously [15,16]. Briefly, CHO cells were grown in 100-mm plastic Petri dishes in Dulbecco's modified Eagle medium containing 10% fetal bovine serum, nonessential amino acids (0.1 mM), penicillin (50 U/ml), and streptomycin (50 μg/ml) in a humidified atmosphere at 37 °C with 5% CO2. For electrophysiological experiments, the cells were seeded on glass coverslips (Carolina Biological Supply Company, Burlington, NC) and transfected the next day using Lipofectamine 2000 (Invitrogen, Milan, Italy). A plasmid encoding for the Enhanced Green Fluorescent Protein (Clontech, Palo Alto, CA) was used as a transfection marker. Total cDNA in the transfection mixture was kept constant at 4 μg. Macroscopic currents from transiently transfected CHO cells were recorded at room temperature (RT, 20-22 °C) 1 day after transfection, with an Axopatch 200A amplifier (Molecular Devices, Union City, CA) using the whole-cell configuration of the patch-clamp technique, with glass micropipettes of 3-5 MΩ resistance. The extracellular solution contained (mM): 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 (mM): 140 KCl, 2 $MgCl_2$, 10 EGTA, 10 HEPES, 5 Mg-ATP, 0.25 cAMP, pH 7.3-7.4 with KOH. The pCLAMP software (version 10.2; Molecular Devices) was used for data acquisition and analysis. Linear cell capacitance (C) was determined by integrating the area under the whole-cell capacity transient, evoked by short (5-10 ms) pulses from -80 to −75 mV with the whole-cell capacitance compensation circuit of the Axopatch 200A turned off. To generate conductance-voltage (G/V) curves, the cells were held at -80 mV and then depolarized for 1.5 sec from -80 to +40 mV in +10 mV increments, followed by an isopotential pulse at 0 mV of 800 ms duration. The current values recorded at the beginning of the 0-mV pulse were normalized and expressed as a function of the preceding voltages. The data were fit to a Boltzmann equation of the following form: $y = \max/(1 + \exp[V_{½} - V)/k)$, where V is the test potential, $V_{½}$ is the half-activation potential, and k is the slope factor. In the experiments with retigabine (Valeant Pharmaceuticals, Aliso Viejo, CA) or gabapentin (Sigma, Milan, Italy) currents were activated by 3-s voltage ramps from -80 mV to 0/+40 mV at 0.1-Hz frequency.

2.4. Neuronal cells preparation, transfection and functional analysis

The procedures involving animals were conducted according to European (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987) and Italian (D.L. n. 26/2014) laws and policies and approved by the University of Milano-Bicocca Animal Care and Use Ethic Commitee (n.02/2019) and by the Italian Ministry of Health (DGSAF 0017710-P-26/07/2017).

Cortical neural cells were isolated from neonatal rats (CD rat, Charles River) between the 2nd and the 3rd postnatal day (P2/3) and cultured in p35 dishes coated with poly-D-lysine in a controlled environment (5% CO_2, 37 °C). Cell medium was composed by Neurobasal A culture medium (Thermo Fisher Scientific) supplemented with B27 (Thermo Fisher Scientific), glutamine (1 mM; Euroclone), β-FGF (10 ng/mL; Thermo Fisher Scientific), penicillin G (50 U/mL; Euroclone) [15]. The day after isolation, cells were transfected with KCNQ2 and KCNQ3 cDNAs (2 μg total cDNA) at a 1:1 ratio (to mimic the genetic balance of normal individuals), using Lipofectamine2000 (Thermo Fisher Scientific) following manufacturer instructions [17]. In order to mimic the genetic balance of affected individuals KCNQ3 was co-transfected with KCNQ2 + mutant KCNQ2 at a 1:0.5:0.5 ratio. Non transfected cells were used as negative control. 48 h after transfection, whole-cell patch-clamp experiments were performed at RT. Cell medium was replaced with an extracellular solution (in mM): 129 NaCl, 35 glucose, 10 HEPES, 3 KCl, 1.8 $MgSO_4$, 1.6 $CaCl_2$ (pH 7.4 with NaOH). Pipettes were pulled to a 4-6 MΩ resistance (Model P-97 Sutter Instruments) and filled with an intracellular solution containing (in mM): 120 K^+-gluconate, 20 phosphocreatine, 15 KCl, 10 HEPES, 2 $MgCl_2$, 2 Na_2ATP, 0.2 EGTA, 0.2 NaGTP, 0.1 leupeptine (pH 7.2 with KOH) [15]. When the whole-cell configuration was obtained, the same depolarizing steps protocol reported above for CHO cells was applied. Current density was obtained dividing the current amplitude measured at each test voltage by the cell capacitance (pA/pF). Data were acquired with a Multiclamp 700B amplifier and Digidata 1440A (Axon Instruments, Molecular Device) and pClamp software (version 10.3; Molecular Devices) and analyzed with Clampfit software (version 10.3; Molecular Devices).

2.5. Statistics

Each data point is the mean ± SEM of at least four determinations, each performed in at least two or three separate experiments. Statistically significant differences were evaluated with the Student's t-test or with the ANOVA followed by the Student-Newman-Keuls test, with the threshold set at $p < 0.05$ and indicated by an asterisk in the Figures and in the Table.

3. RESULTS

3.1. Case description

The patient, now aged 9 years, is the first child of non-consanguineous healthy parents; the girl was born after an uneventful pregnancy and delivery. No familiar history of epilepsy was reported. On the second day of life, a focal epileptic status marked the onset of epilepsy. According to clinical records, seizures were characterized by tonic posturing, head rotation, nystagmus, flushing, crying, and laryngeal stridor. The epileptic status ceased after intravenous phenytoin; however, seizures persisted, refractory to the different therapeutic trials with

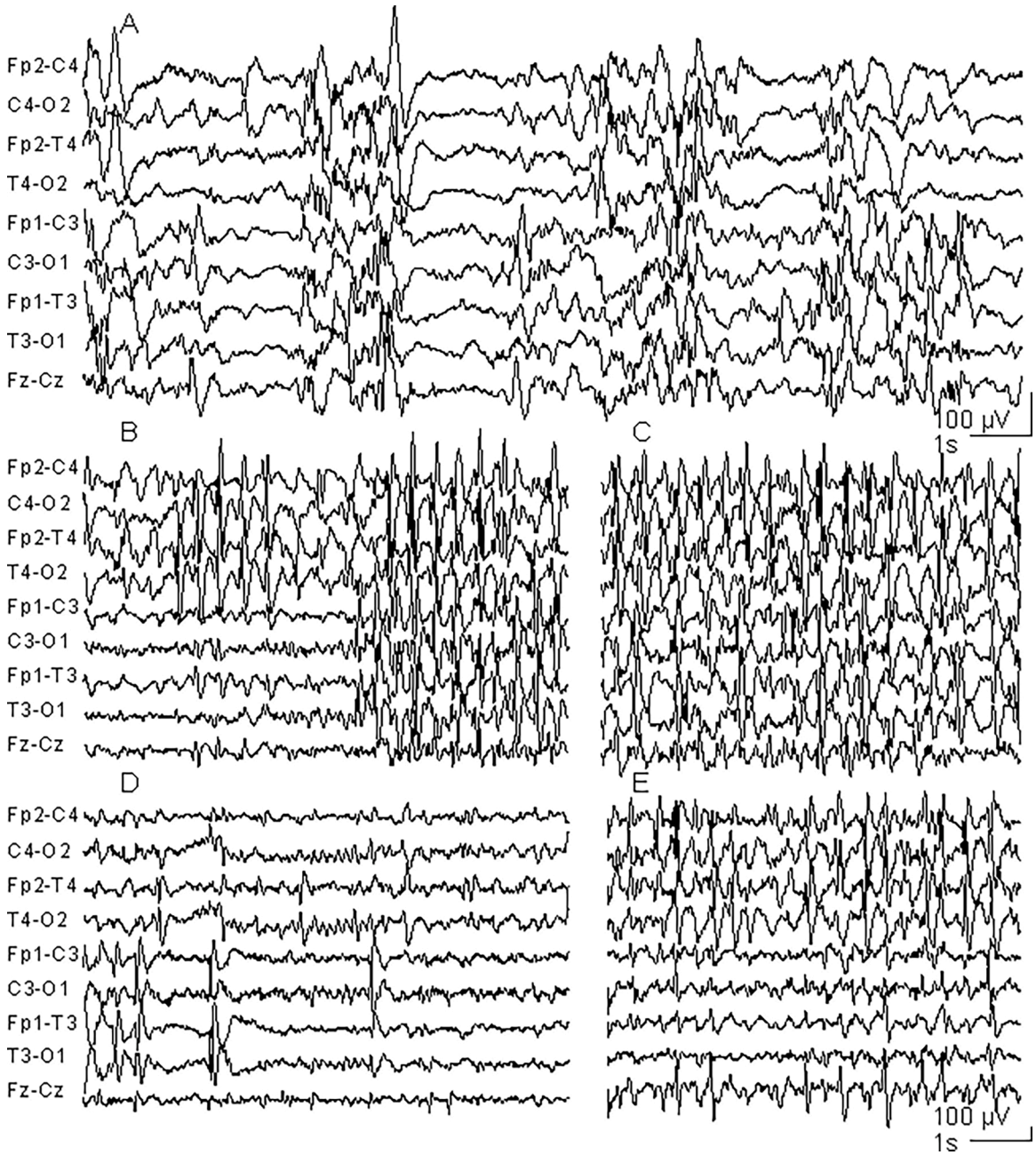

Fig. 1. EEG samples of the patient carrying the Kv7.2 G310S mutation.
At the age of two months, the EEG showed a complete disorganization of the background activity, with burst-suppression pattern (A). At the age of eight years, before GBP treatment, the EEG during wakefulness was characterized by continuous spikes with alternate prevalence of side (B) and spread bilaterally during sleep (C). Following GBP introduction, there was a significant decrease of the epileptiform activity and no seizures were recorded. In particular, the epileptiform activity with focus on the right centro-parietal and temporal areas was reduced during wakefulness (D), with less tendency to spread during sleep (E). Notably, during GBP treatment, a better organization of background activity during wakefulness was observed.

phenobarbital, phenytoin, levetiracetam, vigabatrin and pyridoxine.

At our first observation, at two months of age, the EEG was dominated by a burst-suppression pattern (Fig. 1A) and recurrent seizures with multifocal onset (Supplementary Fig. 1). The electro-clinical picture, characterized by focal seizures and brief, repetitive tonic seizures during sleep and by a burst-suppression EEG pattern, was consistent with the diagnosis of EIEE (Early Infantile Epileptic Encephalopathy), formerly termed Ohtahara syndrome. An extensive diagnostic work-up, including metabolic screening, and CGH array was unrevealing. Brain MRI performed at 2 days (and repeated at 15 days and 3 years of life) failed to identify specific changes in signal intensity in the basal ganglia and in the thalamus, but showed the presence of polymicrogyric cortex in the right fronto-partietal-insular region, which however did not explain the severe clinical course and the EEG pattern (Supplementary Fig. 2). During follow-up, serial EEGs consistently showed subcontinuous multifocal epileptiform abnormalities during wakefulness and sleep. During each EEG session, which lasted at least 90 minutes, at least two seizures during sleep were recorded (Fig. 1B-C). The neurologic evolution was characterized by severe psychomotor delay, absent speech, apostural tetraparesis, and strabismus. The severe clinical course and the presence of seizures which persisted despite treatment with valproate (40 mg/kg) and levetiracetam (60 mg/kg), suggested the need for a diagnostic reevaluation. In particular, genetic investigation was deemed necessary.

3.2. Genetic results

NGS screening revealed a novel variant (c.928 G > A) in *KCNQ2* arising *de novo* in the patient. This DNV is absent in population genetic databases, a result consistent with its potential pathogenetic role in the affected individual. The c.928 G > A DNV affects the first nucleotide of KCNQ2 exon 7, possibly leading to splicing alterations. In fact, *in silico* software predictions suggested a possible decrease of the strength of the physiological acceptor site and the activation of a cryptic site located 30 bp downstream (Supplementary Figure 3A). However, PCR transcript analysis from patient-derived lymphoblasts revealed the presence of only a minority (less than 7%) abnormal transcript lacking the first 30 nucleotides of exon 7 (Supplementary Figure 3B), a result consistent with very slight effects on KCNQ2 splicing and suggesting that the herein identified variant was incorporated in most of KCNQ2 mRNAs, leading to the G310S substitution. Notably, the glycine residue at position 310 in the Kv7.2 C-terminal half of S_6 is highly-conserved among voltage-gated potassium channels including Kv7s, and has been previously shown to play a critical role in Kv7.1 channel gating [18] (Fig. 2A-B).

3.3 Functional and pharmacological characterization of the Kv7.2 G310S variant in CHO cells and cortical neurons

CHO cells transiently-transfected with the Kv7.2 plasmid revealed robust voltage-gated outward K^+ currents; no currents were instead detected in cells transfected with Kv7.2 G310S cDNA (Fig. 2C; Table 1), albeit mutant subunits were clearly detectable both in total lysates and in plasma membrane-enriched fractions (*data not shown*). The expression of Kv7.2 G310S subunits together with Kv7.3 subunits alone or with different ratios of Kv7.2 and Kv7.3 subunits, significantly decreased maximal current density. In particular, current density of Kv7.2+Kv7.3-transfected cells (cDNA ratio 0.5 + 1, respectively) was larger than that of cells transfected with Kv7.2+Kv7.2 G310S + Kv7.3 (cDNA ratio 0.5 + 0.5 + 1, respectively), thus suggesting a mutation-induced strong loss-of-function (LoF) effect, with dominant-negative consequences on wild-type channel behavior (Fig. 2D; Table 1). This result is consistent with previously described genotype-phenotype correlations in *KCNQ2* variants found in severely-affected patients [4,5,16].

Expression of wild-type KCNQ2+KCNQ3 plasmids in neonatal rat cortical neurons generated robust outward currents and hyperpolarized the resting membrane potential (RMP) as compared to non-transfected neurons (Fig. 2E-F; Table 1). Notably, similarly to CHO cells at the same cDNA transfection ratio, co-expression of Kv7.2 G310S subunits in neurons together with Kv7.3 or Kv7.2+Kv7.3 subunits decreased maximal current density and prevented RMP hyperpolarization (Fig. 2E-F; Table 1), thus confirming the strong LoF effects caused by Kv7.2 G310S subunits on neuronal I_{KM}. Based on these results, the ability of Kv7 activators to counteract mutation-induced functional effects *in vitro* was tested. RTG (10 μM; Fig. 2G) and GBP (100 μM; Fig. 2H) both potentiated Kv7.2+Kv7.3 and Kv7.2+Kv7.2 G310S + Kv7.3 currents expressed in CHO cells. In particular, RTG-induced current increase was 90.9 ± 8.4% (n = 15) or 125.8 ± 11.1% (n = 16), in Kv7.2+Kv7.3 or Kv7.2+Kv7.2 G310S + Kv7.3 currents, respectively. Instead, current enhancement by 100 μM GBP was 34.3 ± 3.8% (n = 13) or 32.1 ± 8.5%, (n = 4) in Kv7.2+Kv7.3- or Kv7.2+Kv7.2 G310S + Kv7.3-transfected cells, respectively; a lower GBP concentration (10 μM) also potentiated Kv7.2+Kv7.3 or Kv7.2+Kv7.2 G310S + Kv7.3 currents by 16.2 ± 1.4%, n = 11, or 16.4 ± 1.2%, n = 10, respectively (*data not shown*).

3.4. Personalization of therapy

Based on the described *in vitro* data, at the age of nine years, the patient started treatment with GBP, progressively increased until the target dose of 600 mg daily (28 mg/kg; GBP plasma levels: 3.4 mg/L; therapeutic range 2-20 mg/L). Following introduction of GBP, a significant clinical improvement was observed, with the disappearance of seizures, progress in postural control and interaction with the surrounding environment. The disappearance of seizures, reported by parents, was confirmed by the EEG recordings that, for the first time did not show ictal activity (Fig. 1 D-E). The patient has been seizure free for 18 months, leading to the discontinuation of levetiracetam, while valproate was maintained.

4. DISCUSSION

Precision pharmacology is a novel conceptual paradigm that aims to explore and predict the comprehensive effect of a compound based on its ability to act on the intended target, to interfere with the relevant pathophysiological mechanism, thereby impacting clinical outcome [19]. DEEs comprise a heterogeneous group of severe epilepsies characterized by drug-resistant seizures, frequent EEG abnormalities, and developmental slowing or regression. DNVs in several genes, often encoding for ion channel subunits, are frequently identified in DEE patients [20], and the study of the functional changes caused by distinct variants, together with repurposing of drugs counteracting specific mutation-induced functional effects, appears as a promising strategy for precision pharmacology [21,22]. LoF DNVs in the *KCNQ2* gene represent one of the most frequent causes of early-onset DEE [1,7,22]. Although few cases have reported improvement in patients carrying *KCNQ2* LoF DNVs upon treatment with the selective Kv7 activator RTG [7,23], this drug has been recently withdrawn from the market. Recent findings have identified GBP, a widely-used and well-tolerated drug against seizures and pain [7], as a new Kv7 activator [10], thus providing the unique opportunity to use it for the treatment of DEE-affected patients carrying Kv7.2 LoF DNVs.

In the present work, we describe a patient with DEE caused by a novel Kv7.2 LoF DNV. *In vitro* experiments both in CHO cells and primary cortical neurons (where Kv7.2 subunits are physiologically expressed) showed that this mutation prompted strong LoF effects and that both RTG and GBP produced functional effects opposite to those introduced by the mutation, thus counteracting the mutation-induced functional derangement. Prompted by these results, GBP treatment was started at conventional anticonvulsant dosages in our proband. GBP treatment was well tolerated, and led to clinical improvement and disappearance of seizures. GBP plasma levels in the proband (3.4 mg/L) corresponded to a concentration of about 20 μM, and the therapeutic range of GBP (2-20 mg/L) [24] to concentrations of about 10-100 μM. These values are well within those which could be achieved in the brain of rats after intravenous infusion or bolus administration (about 5 mg/L)

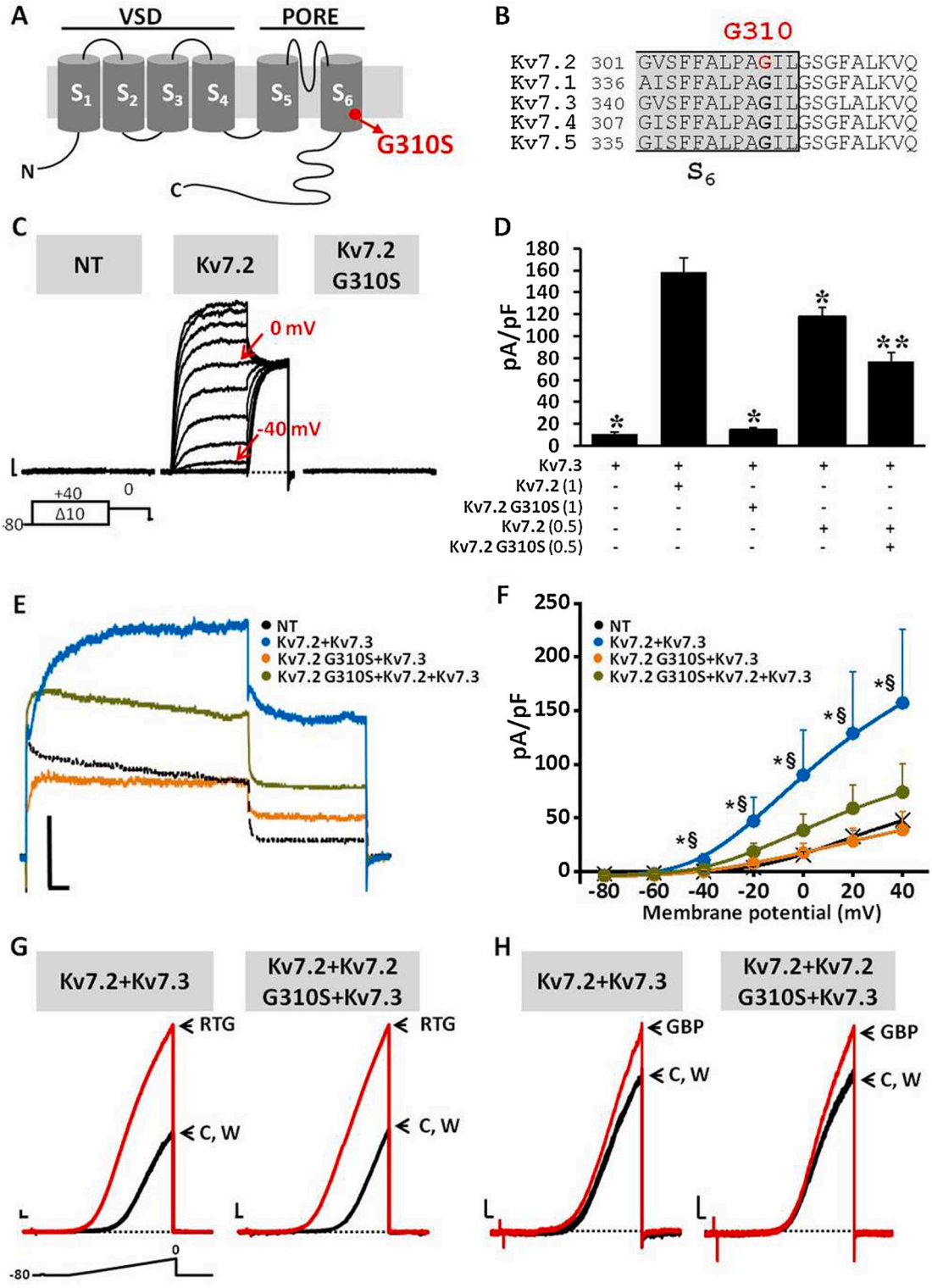

Fig. 2. Functional and pharmacological characterization of the Kv7.2 G310S variant in CHO cells and neurons.
Topological representation of a Kv7.2 subunit and localization of the mutation herein investigated. (B) Partial sequence alignment of Kv7 members; the G310 residue is indicated in bold. (C) Macroscopic currents measured upon application of the voltage protocol shown below in cells expressing the indicated channels; NT = non transfected cells. Current scale: 200 pA; time scale: 100 ms. The arrows indicate the current responses at -40 mV or 0 mV. (D) Maximal current densities in cells transfected with the indicated cDNA constructs; *$p < 0.05$ vs Kv7.2+Kv7.3 (1:1); **$p < 0.05$ vs Kv7.2+Kv7.3 (0.5:1). (E) Current traces at +40 mV from neurons transfected with the indicated cDNA constructs. Current scale: 20 pA/pF; time scale: 100 ms. (F) Maximal current densities in neurons transfected with the indicated cDNA constructs. *$p < 0.05$ vs non transfected neurons (NT); §$p < 0.05$ vs Kv7.2 G310S + Kv7.2+Kv7.3 and Kv7.2 G310S + Kv7.3. (G) Representative current responses to voltage ramps from -80 mV to 0 mV from the indicated channels recorded in control solution (C), after 2 min-perfusion with 10 μM RTG (red traces), and upon drug washout (W). Current scale: 100 pA; time scale: 200 msec. (H) Representative current responses to voltage ramps from -80 mV to 0 mV from the indicated channels recorded in control solution (C), upon 2 min-perfusion with 100 μM gabapentin (GBP), and upon drug washout (W). Current scale: 200 pA; time scale: 200 msec.

Table 1
Functional properties of channels incorporating Kv7.2 G310S subunits expressed in CHO cells or in primary rat cortical neurons

	CHO CELLS				NEURONS				
Experimental Group (cDNA transfection ratio)	**n**	**pA/pF** (@ 0 mV)	**$V_{½}$** (mV)	***k*** (mV per *e*-fold)	**n**	**pA/pF** (@+40 mV)	**$V_{½}$** (mV)	***k*** (mV per *e*-fold)	**V_{REST}** (mV)
NT	4	0.8 ± 0.1	-	-	38	48.8 ± 8.7	−6.8 ± 9.9	-	−41.9 ± 2.4
Kv7.2	10	31.7 ± 4.4	−25.2 ± 0.8	10.4 ± 0.7	-	-	-	-	-
Kv7.2 G310S	10	1.9 ± 0.2*	-	-	-	-	-	-	-
Kv7.3	12	11.2 ± 1.5	−38.5 ± 0.5*	5.3 ± 0.4*	-	-	-	-	-
Kv7.2+Kv7.3 (1:1)	15	158.5 ± 12.7*	−26.2 ± 0.5	9.9 ± 0.4	18	157.2 ± 68.9$^{\#}$	−34.4 ± 9.7$^{\#}$	15.04 ± 4.8	−52.8 ± 1.7$^{\#}$
Kv7.2+Kv7.3 (0.5:1)	21	117.5 ± 9.1**	−29.8 ± 0.5	9.7 ± 0.4	-	-	-	-	-
Kv7.2 G310S + Kv7.3 (1:1)	14	14.3 ± 2.1**	−26.1 ± 0.9	9.9 ± 0.8	22	39.0 ± 16.9	−35.6 ± 23.6	16.3 ± 5.5	−37.9 ± 3.0
Kv7.2+Kv7.2 G310S + Kv7.3 (0.5:0.5:1)	24	77.2 ± 7.8**	−26.4 ± 0.4	9.0 ± 0.3	24	74.2 ± 26.3	−26.8 ± 9.1	14.2 ± 3.6	−42.5 ± 4.0

*=p<0.05 versus Kv7.2; **=p<0.05 versus Kv7.2+Kv7.3; $^{\#}$=p<0.05 versus all other experimental groups in neurons.

[25] and which are able to activate Kv7.2/Kv7.3 channels *in vitro* [10], as also herein reported, thus suggesting that GBP-induced Kv7 channels activation may contribute to the drug-induced clinical improvement observed in our patient.

A final comment relates to the presence of focal polymicrogyria in our patient. The association of cortical malformations and monogenic epilepsies (e.g. SCN1A, STXBP1, PCDH19) has been already reported [26]. Discussing about a possible pathogenic link between the structural abnormalities and channelopathies is beyond the scope of our study. Nevertheless, we would like to underscore that the inconsistency between the clinical syndrome and the most obvious diagnostic finding (in our case focal polymicrogyria at early MRI) should prompt the search for alternative cause that, as in our case, may even lead to a personalized treatment. No specific changes in signal intensity in the basal ganglia and in the thalamus were noted in our patient; noteworthy, brain MRI performed during the first year of life revealed variable T1 and T2 hyperintensities in these same regions in only 6 of 11 patients with KCNQ2 DEE [23].

Altogether, our data provide the first clinical evidence favoring the use of GBP as a personalized treatment of DEE-affected patients caused by Kv7.2 LoF DNVs. Future studies are needed to confirm the efficacy of GBP in larger cohorts of patients with DEE due to Kv7.2 and Kv7.3 LoF DNVs.

Author Contributions

MVS, EF, JCD, and MT: *conception and design of the study; MVS, EF, PA, IR, IM,* AB, CM, FR, LC, CV, RS, TG, BC, GM, CG, AL, GL: *acquisition and analysis of data; MVS, EF, IR, JCD, MT, TG: drafting a significant portion of the manuscript or figures.* All Authors approved the final draft of the manuscript. 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.105200.

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