Journal of Human Genetics (2017) 62, 185–189

www.nature.com/jhg

ORIGINAL ARTICLE

Clinical and genetic features of 13 Spanish patients with *KCNQ2* mutations

Montesclaros Hortigüela[1], Ana Fernández-Marmiesse[2], Verónica Cantarín[1], Sofía Gouveia[2], Juan J García-Peñas[1], Carmen Fons[3], Judith Armstrong[4], Desirée Barrios[5], Felícitas Díaz-Flores[6], Pilar Tirado[7], María L Couce[2,8] and Luis G Gutiérrez-Solana[1]

The *KCNQ2* gene codifies a subunit of the voltage-gated potassium M channel underlying the neuronal M-current. Classically, mutations in this gene have been associated with benign familial neonatal seizures, however, in recent years *KCNQ2* mutations have been reported associated to early-onset epileptic encephalopathy. In this work, detailed familiar, clinical and genetic data were collected for 13 *KCNQ2*-positive patients revealed among a cohort of 80 epileptic pediatric probands from Spain who were analyzed through a targeted next-generation sequencing assay for 155 epilepsy-associated genes. This work shows for the first time the association between *KCNQ2* mutations and startle attacks in 38% of patients, which opens the possibility to define electroclinical phenotypes associated to *KCNQ2* mutations. It also demonstrates that *KCNQ2* mutations contribute to an important percentage of Spanish patients with epilepsy. The study confirm the high genetic heterogeneity of this gene with 13 different mutations found, 10 of them novel and the better outcome of patients treated with sodium channel blockers.
Journal of Human Genetics (2017) **62,** 185–189; doi:10.1038/jhg.2016.104; published online 18 August 2016

INTRODUCTION

A molecular diagnosis in the early-onset epileptic encephalopathies (EOEEs) often has implications for management and prognosis.[1,2] Mutations in *Kv7.2* (*KCNQ2*) and *Kv7.3* (*KCNQ3*) genes, encoding for voltage-gated potassium channel subunits underlying the neuronal M-current (IKM),[3] are responsible for early-onset epileptic diseases with a widely diverging phenotypic presentation.[4] The M channel is a slowly activating and deactivating potassium conductance that has a critical role in determining the subthreshold electroexcitability of neurons as well as the responsiveness to synaptic inputs. Dominant mutations in this channel were first described in 1998 associated to benign familial neonatal epilepsy (BFNS; OMIM:121200) with a favorable prognosis.[4–6] In stark clinical contrast, *KCNQ2* mutations are also associated with EOEE, which has been called *KCNQ2* encephalopathy (OMIM 613720).[7] Patients with *KCNQ2* encephalopathy display tonic seizures 2–3 days after birth with EEG characterized by suppression–burst patterns or multifocal epileptic activity and have poor neurological outcome.[8–10] After the first EOEE-associated *KCNQ2* mutation reported,[11] more mutations in *KCNQ2* had been described subsequently, most of them *de novo*. Now, it has been shown as one of the most common causes of EOEE including the Ohtahara syndrome.[7–18]

In this work, we aim to show the clinical, electrophysiological and genetic features from 13 KCNQ2-positive Spanish patients.

MATERIALS AND METHODS

This study has been approved by the ethics committee from Hospital Universitario Niño Jesús (Madrid, Spain). Detailed familiar and clinical information including age of onset, perinatal data, familiar history, initial and evolutionary seizures semiology, current seizure status, initial and evolutionary psychomotor development, ictal EEG and MRI findings, treatment over time and response to this, were collected for 13 patients with 13 different *KCNQ2* mutations revealed through a targeted NGS-based panel for epilepsy genes. Informed consent was obtained from the parents of all the patients included in the study.

This cohort of KCNQ2 patients were extracted from 80 probands recruited from different Spanish Neuropediatric Units who experienced seizures of unknown etiology and studied through a custom-targeted NGS panel encompassing 155 epilepsy-associated genes to achieve a molecular diagnosis. A custom Sure Select oligonucleotide probe library was designed to capture the coding regions of these epilepsy-associated genes and requested to Agilent Technologies (Santa Clara, CA, USA). Captured fragments were sequenced in pair-end 100-base mode using Miseq Illumina platform from Illumina (San Diego, CA, USA). Reads were aligned to the reference genome GRCh37 with BWA v0.7.5a.[19] Varscan and SAMtools v0.1.19 were the variant detection software used,[20,21] and Annovar for annotation.[22] Prioritization was based on frequency of variants detected given in 1000 genomes, dbSNP and our in-house

[1]Unit of Child Neurology, Department of Pediatrics, Hospital Infantil Universitario Niño Jesús de Madrid, Madrid, Spain; [2]Unit of Diagnosis and Treatment of Inborn Errors of Metabolism (UDyTEMC), Health Research Institute of Santiago de Compostela (IDIS), Galicia, Spain; [3]Unit of Child Neurology, Department of Pediatrics, Hospital Sant Joan de Deu, Barcelona, Spain; [4]Biochemical, Genetics and Rett Unit, Laboratory Department, Hospital Sant Joan de Déu, Esplugues, Barcelona, Spain; [5]Department of Pediatrics. Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain; [6]Molecular Genetics Unit, Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain; [7]Unit of Child Neurology, Department of Pediatrics, Hospital Universitario la Paz, Madrid, Spain and [8]Centro de Investigación Biomédica de Enferemedades Raras (CIBERER), Instituto de Salud Carlos III, Spain
Correspondence: Dr Montesclaros Hortigüela Saeta, Hospital Infantil Universitario Niño Jesús de Madrid, Avenida Menéndez Pelayo, No 65, 28009 Madrid, Spain.
E-mail: montesclaroshs@gmail.com
Received 22 March 2016; revised 22 June 2016; accepted 23 June 2016; published online 18 August 2016

Table 1 Clinical features of 13 Spanish patients positive for *KCNQ2* mutations

	Age at onset (age at last examination)	*Seizure semiology (initial)*	*Seizure semiology (evolution)*	*Initial psychomotor development*	*Diagnosis*	*Brain MRI*	*EEG core activity*	*EEG intercritical abnormalities*	*Present crisis*	*Current status and comorbidity*	*Family history*
P1	10 h (12 m)	TS Startle episodes	No	Early PD with spastic CP AH	EOEE (Ohtahara type)	Normal	SBP	Multifocal epileptiform abnormalities	SF	Spastic CP Severe PD	Negative
P2	48 h (6 y)	FS with apnea Startle episodes	ES Asymmetric TS	Early PD	EOEE	Cortical–subcortical atrophy with altered white matter associated with a thin corpus callosum	Hypsarrhythmia	Multifocal epileptiform abnormalities	ES Asymmetric TS	Severe CI Severe PD	Negative
P3	8 d (10 y)	TS	MS, TCS	Early PD	Epilepsy in a Rett-like patient	Normal	Acceptably organized for age	Multifocal epileptiform abnormalities	SF	Rett-like phenotype	Negative
P4	<24 h (25 m)	TS with apnea	TCS FS	Early PD Severe AH	EOEE (Ohtahara type) Multifocal focal epilepsy	(3 weeks): delay of myelination. (12 months): slight delay of myelination	SBP	Multifocal epileptiform abnormalities	SF	Severe PD	Negative
P5	<12 h (20 m)	MS with apnea Startle episodes.	Axial TS ES	Early PD Severe AH	Myoclonic EOEE (Aicardi type) evolving to West syndrome	Normal	SBP evolving to hypsarrhythmia	Multifocal epileptiform abnormalities	Multifocal MS ES	Dyskinesia Acquired microcephaly	NA[a]
P6	<8 h (42 m)	TS with apnea Startle episodes FS	Alternating focal TS	PD since 6th month Severe AH	Focal epilepsy Startle disease Hyperekplexia suspicion	Normal	Acceptably organized for age	Well-defined epileptiform abnormalities in both regions with bilateral fronto-central spikes	SF	Ataxia Postural and intentional tremor Acquired microcephaly	Negative
P7	<12 h (26 m)	TS with apnea	ES	Early PD Severe AH	EOEE (Ohtahara type)	Normal	SBP evolving to hypsarrhythmia	Multifocal epileptiform abnormalities	ES	Dyskinesia ASD Acquired microcephaly	Positive[b]
P8	<24 h (24 m)	TS with apnea Startle episodes	Asymmetric TS	Early PD Severe AH	EOEE (Ohtahara type)	Normal	SBP	Multifocal epileptiform abnormalities	SF	Dyskinesia	Positive[c]
P9	2 d (24 m)	TS	Asymmetric TS, ES, Episodes of lethargy and stupor	Early PD Severe AH	EOEE	Normal	Hypsarrhythmia	Epileptiform abnormalities predominantly in the right frontal region	ES	PD	Positive[d]
P10	3 d (4 y)	Eye rolling, joint stiffness and upper limb clonus	No	Normal	BFNS	Normal[e]	Normal	Normal	SF	Normal	Positive[f]
P11	5 d (24 m)	FS	No	Normal PD with minimal axial dystonia and exalted ROT	BFNS	Normal	Normal	Initially intercritical paroxysmal abnormalities on both frontal regions of low persistent, with normal subsequent records	SF	Normal	Positive[f]
P12	3 d (36 m)	FS	No	PD, AH, Exalted ROT Subsequently DPM and normal physical examination	BNS	Normal	Initially: asymmetric background activity with higher voltage and fast activity more intense in the right hemisphere with bilateral acute spikes. Currently: normal	Diffuse spike and slow waves Currently normal	SF	Normal	Negative
P13	48 h (43 m)	FS with apnea	No	Normal	BNS	Normal	Normal	Normal	SF	Normal	Negative

Abbreviations: AH, axial hypotonia; ASD, autism spectrum disorder; BNS, benign neonatal seizures; CI, cognitive impairment; CP, cerebral palsy; EE, epileptic encephalopathy; EEG, electroencephalography; EOEE, early-onset epileptic encephalopathy; ES, epileptic spasms; FS, focal seizures; MS, myoclonic seizures; PD, psychomotor delay; SBP, supression–burst pattern; SF, seizures free (patient without seizures since the introduction of a new drug to the present (last examination)); TCS, tonic–clonic seizures; TS, tonic seizures.

[a]NA, not available (born from donated gametes).

[b]Paternal uncle had early West syndrome (3 months) and died at 20 months from pneumonia.

[c]Father febrile seizures.

[d]Father diagnosed with epilepsy at 15 years of age, treated with carbamazepine. Free seizures at present.

[e]Brain ultrasound: peripheral hyperechoic white matter lesions in thalamus.

[f]Mutation found in different family members showing cosegregation.

Table 2 Antiepileptic drugs administrated to *KCNQ2*-positive patients

Patient	*Initial*	*During evolution*	*Others*	*Current treatment*
P1	PB	LEV, MDZ, VPA, PHT, TPM, VGB, CNZ	CoF, PRD	**CBZ** (seizure free)
P2	PB + PRX	VPA, VGB, ZNS, CLB, CBZ, LEV	PRX	ZNS, retreating VPA, starting with **CBZ**
P3	PB	LEV, LTG		**LTG** (seizure free)
P4	PB	LEV, PHT, TPM, OXC	CoF	**OXC**+TPM (seizure free)
P5	PB	PB, MDZ, LID,LEV, VPA, CNZ, VGB, ZNS	CoF, ACTH, PRD, KD induced coma with high doses of PB+MDZ	VPA+VGB+LEV+KD
P6	PB	PB, CNZ, VPA, LEV, MDZ, OXC, AZM, PHT, LCM, CLB, CBZ	CoF	VPA+**CBZ+LCM** (seizure free)
P7	PB	PB, MDZ, LEV, PHT, ZNS, VPA, VGB, CLB	CoF, KD	VGB+ZNS
P8	PB	VPA,OXC		PB+VPA+**OXC** (seizure free)
P9	LEV	LEV, PB, PHT, CNZ, VGB,GBP, TPM, PRD	PRD	LEV+GBP+TPM+VGB+PB+PRD
P10	PB	VPA		No treatment
P11	LEV			No treatment
P12	MDZ	MDZ, LID, LEV, TPM	CoF	No treatment
P13	PB	LEV, CBZ		**CBZ** (seizure free)

Abbreviations: AZM, acetazolamide; CBZ, carbamazepine; CLB, clobazam; CNZ, clonazepam; CoF, cofactors (biotin, folate); CTH, adrenocorticotropic hormone; GBP, gabapentine; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; LID, lidocaine; LTG, lamotrigine; MDZ, midazolam; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; PRD, prednisone; PRX, pyridoxine; TPM, topamax; VGB, vigabatrin; VPA, valproic acid; ZNS, zonisamide.
The bold entries represent inhibitors of sodium channels.

population database (collected sequencing data from 80 patients and 120 controls from Spanish origin). Sanger dideoxy sequencing method was used to perform familiar studies of KCNQ2 mutations detected in the patients. Missense mutations were evaluated with CONDEL (http://bg.upf.edu/fannsdb/).[23] This tool integrates the output of five computational tools (SIFT, Polyphen2, MAPP, LogR Pfam E-value and MutationAssessor) aimed at assessing the impact of non-synonymous SNVs on protein function. To do this, it computes a weighted average of the scores of these tools.

The novel KCNQ2 mutations have been submitted to the ClinVar (https://submit.ncbi.nlm.nih.gov/subs/variation_submission/SUB1205799/overview) and to the Locus Specific (http://databases.lovd.nl/shared/users/01470) databases.

RESULTS

Clinical findings

From the 13 patients studied, nine of them (P1–P9) presented a severe electroclinical phenotype (EOEE), whereas the remaining four patients (P10–P13) were cataloged as benign neonatal seizures (BNS; Table 1). None of the cases, except P1, showed a prominent perinatal history. In this patient, it was observed perinatal asphyxia data (presence of meconium and the need of pocket resuscitation mask) without showing hypoxic-ischemic encephalopathy in the following hours.

Five patients had positive family history, until third-degree relatives, of epilepsy. All children had seizures onset in the neonatal period. The successive treatments applied are summarized in Table 2.

From the nine patients with *KCNQ2*-EOEE, seven debuted with tonic seizures, in six patients, seizures were accompanied by autonomic features characterized by brief respiratory pauses (apnea episodes). In the evolution, four presented epileptic spasms, and five showed exaggerated startle attacks. Five patients presented suppression–burst pattern in EEG, with four of them cataloged as Ohtahara syndrome and one with early myoclonic epileptic encephalopathy of Aicardi. Four patients developed EEG compatible with hypsarrythmia, two of them with an initial suppression–burst pattern. All the patients developed early psychomotor delay and presented intercritical multifocal epileptic activity during evolution. In two patients, MRI showed an altered pattern: P2, a cortico-subcortical atrophy; and P4, a slight delay in the myelination. Seven patients showed hypotonia. The mean follow-up period of the patients was 1–10 years and five are currently seizure free.

All BNS cases, with a mean follow-up period of 2–4 years, showed normal psychomotor development. P10 showed hyperechoic peripheral white matter lesions in the thalamus, presenting later a normal MRI. Three of them showed focal seizures at the onset and two showed epileptiform discharges in initial EEG and gradually returned toward normal subsequently.

Genetic findings

Thirteen different *KCNQ2* mutations, 12 missense and one frameshift, were found in the 13 patients recruited in this work, nine EOEE-associated, two BNS-associated and two BFNS-associated (Table 3). Familiar genetic study was performed in all the cases. All the mutations found in EOEE cases and two BNS-associated mutations were *de novo* or the result of parental mosaicism, as it was not detected in DNA extracted from the whole blood of their parents. However, it is important to remark that in three of the EOEE patients, a positive family history for epilepsy was found. In two of the four BNS patients, the mutation was found in different family members with BNS showing cosegregation. Ten mutations were found to be novel, and five caused alteration of amino acid residues at which different missense changes had been reported previously in patients with BFNS and EOEE (Table 3). Mutations p.A265T and p.R553W caused alteration of amino acid at which two different missense changes have been previously described, one of them BFNS-associated and the other EOEE-associated. The mutation c.388G>A found in P8 takes place in the first nucleotide of exon 3, but this variant do not seem to cancel the consensus sequence required for the adequate splicing of such exon, according to the prediction made with human splicing program finder (HSF v.3.0). All the mutations have CONDEL values between 0.57 and 0.74, and all are predicted to be pathogenic. Amino acids 130, 201, 265, 268, 274, 284, 294, 306, 553 are highly conserved during evolution as it is shown by a SIFT value of zero, and the amino acids changes considered more drastic are R201C, T274M, R553W, R553L, R553Q.

DISCUSSION

Ages of onset and EEG findings are similar to those previously described.[7,8,10,14,15,17,24] Nevertheless, we noted that five patients

Table 3 Summary of the variants identified in the *KCNQ2* gene in 13 Spanish patients

Patient	*Nt change*	*Aa change*	*CONDEL*[a]	*Exon*	*Inheritance*	*Family history*	*Phenotype*	*Protein domain*	*Reference*[b] *(associated phenotype)*	*Variants in the same codon leading to other/s amino acid change(reference) (phenotype)*
P1	c.881C>T	p.A294V	0.59	E6	*De novo*	Negative	EOEE	Transmembrane S6	Kato *et al.* (2013) (EOEE)	p.A294G (Steinlein *et al.*, 2007) (BNFS1)
P2	c.850T>G	p.Y284D	0.63	E6	*De novo*	Negative	EOEE	Pore-forming; H5	Novel	p.Y284C (Singh *et al.*, 1998) (BNFS1)
P3	c.917C>T	p.A306V	0.65	E6	*De novo*	Negative	EOEE	Transmembrane S6	Novel	p.A306T (Singh *et al.*, 1998) (BNFS1)
P4	c.803T>C	p.L268P	0.68	E5	*De novo*	Negative	EOEE	Pore-forming; H5	Novel	—
P5	c.601C>T	p.R201C	0.72	E4	*De novo*	NA[c]	EOEE	Transmembrane S4	Miceli *et al.* (2015) (EOEE)	—
P6	c.943G>C	p.G315R	0.61	E7	*De novo*	Negative	EOEE	Cytoplasmic	Novel	—
P7	c.793G>A	p.A265T	0.57	E5	*De novo*	Positive[d]	EOEE	Pore-forming; H5	Novel	p.A265V (Kato et al 2013) (EOEE) p.-A265P (Weckhuysen et al., 2012) (BNFS1)
P8	c.388G>A	p.E130K	0.67	E3	*De novo*	Positive[e]	EOEE	Transmembrane S2	Novel	—
P9	c.821C>T	p.T274M	0.74	E6	*De novo*	Positive[f]	EOEE	Pore-forming; H5	Novel	—
P10	c.775G>T	p.D259Y	0.61	E5	Inherited from father	Positive[g]	BFNS	Extracellular	Novel	—
P11	c.2127delT	p.V710Sfs[a] 220	Not applicable	E17	Inherited from mother	Positive[g]	BFNS	Cytoplasmic	Novel	—
P12	c.319C>T	p.L107F	0.65	E2	*De novo*	Negative	BNS	Transmembrane S1	Novel	—
P13	c.1657C>T	p.R553W	0.67	E15	*De novo*	Negative	BNS	Cytoplasmic	Kato *et al.* (2013) (EOEE)	p.R553L (Kato et al 2013) (EOEE) p.-R553Q (Mowlan et al.2001) (BNFS1)

[a]A mutation is considered deleterious by the software if the score is above 0.52.
[b]For previously reported variants.
[c]NA, not available (born from donated gametes).
[d]Paternal uncle had early West syndrome (3 months) and died at 20 months from pneumonia.
[e]Father febrile seizures.
[f]Father diagnosed with epilepsy at 15 years of age, treated with carbamazepine. Free seizures at present.
[g]Mutation found in different family members showing cosegregation.
Mutations are numbered based on the reference sequence NM_172107.2.

(38%) showed exaggerated startle attacks (nonepileptic tonic postures that appear as a result of different sensorial stimulus like sound or touch), which had never been published before in these patients. Only Allen *et al.*[14] described nonepileptic extensor jerks in one case. Although seizures may be similar in both phenotypes,[10,14] neurological status and abnormal EEG pattern allow to distinguish them (EEOE vs BFNS).[7,11,14] These electroclinical features may suggest the diagnosis of *KCNQ2* encephalopathy.

From the nine patients with *KCNQ2* encephalopathy, five remain seizure free but with severe impairment in psychomotor development, similar to that observed in other series.[14,15,24] All these patients presented initial refractory epilepsy, but five became seizure free when they took inhibitors of sodium channels as it had been previously reported.[7,15,24] Numis *et al.*[24] reported that this beneficial effect seems to be due to the fact that both sodium and potassium channels are linked and located at critical points in the neuronal membrane. Thus, modulation of one of them, can significantly improve the function of the whole channel system. Retigabine has been postulated as an even more effective drug as it increases the potassium current through KCNQ channels;[18,24] however, it was not used in our cohort because of adverse effects.

Mutations in the *KCNQ2* gene was initially discovered in patients with BFNS;[5,6] however, many cases associated with EOEE[7–18] were subsequently recognized. In the literature, it has been estimated that between 5 and 23% of all EOEE cases are secondary to mutations in *KCNQ2*.[7,15,17] In our cohort, mutations in *KCNQ2* accounts for about 16% of pediatric patients with seizures of unknown etiology. The reason why *KCNQ2* mutations can lead to these two markedly different phenotypes is currently unknown. From the occurrence of *KCNQ2* deletions in BFNS, it is known that a total loss of function of one allele leads to the benign phenotype only; therefore, haploinsufficiency cannot explain the more severe *KCNQ2*-EOEE. Even more, all *KCNQ2*-EOEE mutations published so far are missense mutations, but never loss-of-function mutations (always BFNS-associated). A possible explanation for this finding would be that haploinsufficiency may be physiologically reverted as it is shown in BFNS patients meanwhile alleles with missense mutations can be used to form functional channels but with different sensitivities to voltage promoted by the amino acid change. This finding could lead to the development of personalized treatment for *KCNQ2*-EOEE. Therapies using antisense oligonucleotides are being specifically developed for diseases with known dominant-negative mutations. Specifically inhibiting transcription or translation of the mutated *KCNQ2* allele would lead to a loss-of-function situation, mimicking a *KCNQ2* haploinsufficiency, which in turn, is known to lead to the milder BFNS phenotype. Therefore such strategy has the potential to turn a severe EOEE into a benign neonatal epilepsy syndrome.[15]

There has been reported several studies trying to explain the quite different phenotypes associated to *KCNQ2* mutations. First explanation is the extent of the mutation-induced functional K+ channel impairment. Functional studies have revealed that *KCNQ2*-BFNS causing mutations decrease IKM conductance, and a 25% reduction

in Kv7.2 currents appears sufficient to increase neuronal excitability to epileptogenic levels in early infancy;[25] animal models appeared to confirm such conclusion.[26] More dramatic functional deficits have been found in channels carrying mutations associated with more severe epileptic phenotypes.[18,27] With regard to this, it has been shown that two mutations affecting the same positively charged residue in the S4 domain of KV7.2 were found in children affected with BFNS (R213W) or with EOEE (R213Q). Functional studies revealed that both mutations increased cell firing frequency,[27] with the R213Q mutation prompting more dramatic functional changes compared with the R213W mutation. These results suggested that the clinical disease severity may be related to the extent of the mutation-induced functional K+ channel impairment, and set the preclinical basis for the potential use of Kv7 openers as a targeted anticonvulsant therapy to improve developmental outcome in neonates with KV7.2 encephalopathy. Nevertheless, a recent work[28] showed that three mutations placed in the voltage-sensing domain (R144Q, R201C and R201H) found in patients with EOEE stabilized the activated state of the channel, suggesting that a gain-of-function mechanism may also cause *KCNQ2*-EOEE. In brief, depending on the position and the features of the amino acid change, its influence in the voltage sensitivity of such channel will be variable. The change could promote increase and decrease of channel activity and with different intensity levels.

Three patients with supposed *de novo* mutations had positive family history for epilepsy in our cohort. It is important to highlight that a different pattern for heredity must be considered given that mosaicism may have an important role in the genetic burden of neurologic disease.[29,30] Electroclinical pattern and seizures response with inhibitors of sodium channels could be essential for the early diagnosis of KCNQ2 encephalopathy helping a more effective and targeted treatment.

To conclude, it should be noted that the presence of tonic seizures in the neonatal period accompanied by autonomic features, suppression–burst pattern in EEG and exaggerated startle attacks could be essential for suspected diagnosis of KCNQ2-EOEE and begin early treatment with sodium channels inhibitors until genetic confirmation. This work also shows that KCNQ2 mutations contribute to the development of about 16% of total pediatric Spanish patients with seizures of unknown etiology. It also confirms (1) the broader genetic heterogeneity of this gene with 10 novel KCNQ2 mutations found and (2) the two different phenotypes promoted depending of the amino acid change involved, even for the same protein position.

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was financially supported, in part, by a grant from the Spanish ministry of health (Instituto de Salud Carlos III (FIS), Grant No.PI13/2177).

1 Ottman, R., Hirose, S., Jain, S., Lerche, H., Lopes-Cendes, I., Noebels, J. L. *et al.* Genetic testing in the epilepsies—report of the ILAE Genetics Commission. *Epilepsia* **51**, 655–670 (2010).
2 Thomas, R. H. & Berkovic, S. F. The hidden genetics of epilepsy—a clinically important new paradigm. *Nat. Rev. Neurol.* **10**, 283–292 (2014).
3 Wang, H. S., Pan, Z., Shi, W., Brown, B. S., Wymore, R. S., Cohen, I. S. *et al.* KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science* **282**, 1890–1893 (1998).
4 Biervert, C., Schroeder, B. C., Kubisch, C., Berkovic, S. F., Propping, P., Jentsch, T. J. *et al.* A potassium channel mutation in neonatal human epilepsy. *Science* **279**, 403–406 (1998).
5 Charlier, C., Singh, N. A., Ryan, S. G., Lewis, T. B., Reus, B. E., Leach, R. J. *et al.* A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat. Genet.* **18**, 53–55 (1998).
6 Singh, N. A., Charlier, C., Stauffer, D., DuPont, B. R., Leach, R. J., Melis, R. *et al.* A novel potassium channel gene, KCNQ2, is mutated an inherited epilepsy of newborns. *Nat. Genet.* **18**, 25–29 (1998).
7 Kato, M., Yamagata, T., Kubota, M., Arai, H., Yamashita, S., Nakagawa, T. *et al.* Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation. *Epilepsia* **54**, 1282–1287 (2013).
8 Serino, D., Specchio, N., Pontrelli, G., Vigevano, F. & Fusco, L. Video/EEG findings in a KCNQ2 epileptic encephalopathy: a case report and revision of literature data. *Epileptic Disord.* **15**, 158–165 (2013).
9 Saitsu, H., Kato, M., Koide, A., Goto, T., Fujita, T., Nishiyama, K. *et al.* Whole exome sequencing identifies KCNQ2 mutations in Ohtahara syndrome. *Ann. Neurol.* **72**, 298–300 (2012).
10 Weckhuysen, S., Mandelstam, S., Suls, A., Audenaert, D., Deconinck, T., Claes, L. R. *et al.* KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. *Ann. Neurol.* **71**, 15–25 (2012).
11 Dedek, K., Fusco, L., Teloy, N. & Steinlein, O. K. Neonatal convulsions and epileptic encephalopathy in an Italian family with a missense mutation in the fifth transmembrane region of KCNQ2. *Epilepsy Res.* **54**, 21–27 (2003).
12 Borgatti, R., Zucca, C., Cavallini, A., Ferrario, M., Panzeri, C., Castaldo, P. *et al.* A novel mutation in KCNQ2 associated with BFNC, drug resistant epilepsy, and mental retardation. *Neurology* **63**, 57–65 (2004).
13 Dalen Meurs-van der Schoor, C., van Weissenbruch, M., van Kempen, M., Bugiani, M., Aronica, E., Ronner, H. *et al.* Severe neonatal epileptic encephalopathy and KCNQ2 mutation: neuropathological substrate? *Front. Pediatr.* **2**, 136 (2014).
14 Allen, N. M., Mannion, M., Conroy, J., Lynch, S. A., Shahwan, A., Lynch, B. *et al.* The variable phenotypes of KCNQ-related epilepsy. *Epilepsia* **55**, e99–e105 (2014).
15 Weckhuysen, S., Ivanovic, V., Hendrickx, R., Van Coster, R., Hjalgrim, H., Møller, R. S. *et al.* Extending the KCNQ2 encephalopathy spectrum: clinical and neuroimaging findings in 17 patients. *Neurology* **81**, 1697–1703 (2013).
16 Carvill, G. L., Heavin, S. B., Yendle, S. C., McMahon, J. M., O'Roak, B. J., Cook, J. *et al.* Targeted resequencing in epileptic encephalopathies identifies *de novo* mutations in CHD2 and SYNGAP1. *Nat. Genet.* **45**, 825–830 (2013).
17 Milh, M., Boutry-Kryza, N., Sutera-Sardo, J., Mignot, C., Auvin, S., Lacoste, C. *et al.* Similar early characteristics but variable neurological outcome of patients with a de novo mutation of KCNQ2. *Orphanet J. Rare Dis.* **8**, 80 (2013).
18 Orhan, G., Bock, M., Schepers, D., Ilina, E. I., Reichel, S. N., Löffler, H. *et al.* Dominant-negative effects of KCNQ2 mutations are associated with epileptic encephalopathy. *Ann. Neurol.* **75**, 382–394 (2014).
19 Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
20 Koboldt, D. C., Chen, K., Wylie, T., Larson, D. E., McLellan, M. D., Mardis, E. R. *et al.* VarScan: variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics* **25**, 2283–2285 (2009).
21 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N. *et al.* The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
22 Wang, K., Li, M. & Hakonarson, H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* **38**, e164 (2010).
23 González-Pérez, A. & López-Bigas, N. Improving the assessment of the outcome of nonsynonymous SNVs with a consensus deleteriousness score, Condel. *Am. J. Hum. Genet.* **88**, 440–449 (2011).
24 Numis, A. L., Angriman, M., Sullivan, J. E., Lewis, A. J., Striano, P., Nabbout, R. *et al.* KCNQ2 encephalopathy: delineation of the electroclinical phenotype and treatment response. *Neurology* **82**, 368–370 (2014).
25 Jentsch, T. Neuronal KCNQ potassium channels: physiology and role in disease. *Nat. Rev. Neurosci.* **1**, 21–30 (2000).
26 Singh, N. A., Otto, J. F., Dahle, E. J., Pappas, C., Leslie, J. D., Vilaythong, A. *et al.* Mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. *J. Physiol.* **586**, 3405–3423 (2008).
27 Miceli, F., Soldovieri, M. V., Ambrosino, P., Barrese, V., Migliore, M., Cilio, M. R. *et al.* Genotype-phenotype correlations in neonatal epilepsies caused by mutations in the voltage sensor of K(v)7.2 potassium channel subunits. *Proc. Natl Acad. Sci. USA* **110**, 4386–4391 (2013).
28 Miceli, F., Soldovieri, M. V., Ambrosino, P., De Maria, M., Migliore, M., Migliore, R. *et al.* Early-onset epileptic encephalopathy caused by gain-of-function mutations in the voltage sensor of Kv7.2 and Kv7.3 potassium channel subunits. *J. Neurosci.* **35**, 3782–3793 (2015).
29 Milh, M., Lacoste, C., Cacciagli, P., Abidi, A., Sutera-Sardo, J., Tzelepis, I. *et al.* Variable clinical expression in patients with mosaicism for KCNQ2 mutations. *Am. J. Med. Genet. A* **167**, 2314–2318 (2015).
30 Depienne, C., Trouillard, O., Gourfinkel-An, I., Saint-Martin, C., Bouteiller, D., Graber, D. *et al.* Mechanisms for variable expressivity of inherited SCN1A mutations causing Dravet syndrome. *J. Med. Genet.* **47**, 404–410 (2010).