Genotype-phenotype correlations in patients with de novo KCNQ2 pathogenic variants

Federica Malerba, MD, Giulio Alberini, PhD, Ganna Balagura, MD, Francesca Marchese, MD, Elisabetta Amadori, MD, Antonella Riva, MD, Maria Stella Vari, MD, PhD, Elena Gennaro, PhD, Francesca Madia, PhD, Vincenzo Salpietro, MD, PhD, Marco Angriman, MD, Lucio Giordano, MD, Patrizia Accorsi, MD, Marina Trivisano, MD, Nicola Specchio, MD, Angelo Russo, MD, Giuseppe Gobbi, MD, Federico Raviglione, MD, Tiziana Pisano, MD, Carla Marinì, MD, PhD, Maria M. Mancardi, MD, Lino Nobili, MD, PhD, Elena Freri, MD, Barbara Castellotti, MD, Giuseppe Capovilla, MD, Antonietta Coppola, MD, PhD, Alberto Verrotti, MD, Paola Martelli, MD, Francesco Miceli, PhD, Luca Maraglino, PhD, Fabio Benfenati, MD, PhD, Maria R. Cilio, MD, Kathrine M. Johannessen, MD, Rikke S. Møller, PhD, Berten Ceulemans, MD, Carlo Minetti, MD, PhD, Sarah Weckhuysen, MD, PhD, Federico Zara, PhD, Maurizio Taglialetela, PhD, and Pasquale Striano, MD, PhD

Neur Genet 2020;6:e528. doi:10.1212/NXG.0000000000000528

Abstract

Objective

Early identification of de novo KCNQ2 variants in patients with epilepsy raises prognostic issues toward optimal management. We analyzed the clinical and genetic information from a cohort of patients with de novo KCNQ2 pathogenic variants to dissect genotype-phenotype correlations.

Methods

Patients with de novo KCNQ2 pathogenic variants were identified from Italy, Denmark, and Belgium. Atomic resolution Kv7.2 structures were also generated using homology modeling to map the variants.

Results

We included 34 patients with a mean age of 4.7 years. Median seizure onset was 2 days, mainly with focal seizures with autonomic signs. Twenty-two patients (65%) were seizure free at the mean age of 1.2 years. More than half of the patients (17/32) displayed severe/profound intellectual disability; however, 4 (13%) of them had a normal cognitive outcome. A total of 28 de novo pathogenic variants were identified, most missense (25/28), and clustered in conserved regions of the protein; 6 variants recurred, and 7 were novel. We did not identify a relationship between variant position and seizure offset or cognitive outcome in patients harboring missense variants. Besides, recurrent variants were associated with overlapping epilepsy features but also variable evolution regarding the intellectual outcome.

Conclusions

We highlight the complexity of variant interpretation to assess the impact of a class of de novo KCNQ2 mutations. Genetic modifiers could be implicated, but the study paradigms to successfully address the impact of each single mutation need to be developed.
Heterozygous KCNQ2 mutations cause genetic neonatal-infantile epilepsy, ranging from benign familial neonatal epilepsy (BFNE) to severe developmental epileptic encephalopathy (DEE).1 Most KCNQ2 variants associated with BFNE lead to haploinsufficiency,1,2 whereas in patients with KCNQ2-related DEE, de novo mutations are mostly missense,3 usually with dominant negative effect.1,2 Despite the amount of data regarding KCNQ2 pathogenic variants being published or deposited in more general purpose web resources, a description of the clinical phenotype associated with a KCNQ2 de novo variant, with a focus on the degree of cognitive impairment and epilepsy features, is crucial.4,5 We investigated a cohort of patients with de novo KCNQ2 variants to define their clinical features and genotype-phenotype correlations.

Methods

Patients
Children with epilepsy with de novo KCNQ2 variants identified by Sanger sequencing or target resequencing and following American College of Medical Genetics and Genomics classification6 were recruited through a collaboration between different European centers. Clinical and instrumental data at onset and during follow-up were collected from medical charts. Motor and intellectual development were assessed through developmental milestones (eye contact, head control, walking, and speech), neurologic examination, and—when available—developmental quotient.

Standard protocol approvals, registrations, and patient consents
Institutional/regional ethical committee gave approval for research; informed consent was signed by guardians.

Data availability
Patients’ data set and clinical information are summarized in table e-1 (links.lww.com/NXG/A334); further details are available on request.

Structural modeling
We investigated the position of the residues involved in the missense mutations using a tridimensional configuration of the homotetrameric human Kv7.2 (hKv7.2) channel. Each subunit comprises 6 transmembrane (TM) consecutive helices: from the cytoplasmatic N-terminal, the first 4 (S1-S4) serve as the voltage-sensing domain (VSD) due to several positively charged residues in S4; the other 2, S5 and S6, form the pore module, and they are linked by the so-called P-loop, which includes a short P helix and the selectivity filter. Finally, the S6 helix connects with a long C-terminal region in the cytosol. Our model highlights residues in the TM domains, where all the 6 mutations occurring twice in the cohort are located. The structural model is shown in the figure, colored according to the sequence conservation profile calculated with the ConSurf server.7

Statistical analysis
Data were analyzed using the 2-way Student t test. Values reflect the mean, and error bars reflect SEM; p < 0.05. Additional information on data acquisition is in supplementary information, links.lww.com/NXG/A334.

Results

Clinical features
Thirty-four patients (23 females) were included (table e-1, links.lww.com/NXG/A334). Age at the last follow-up ranged from 4 months to 13 years (mean: 4.7 ± 3.7 years). All patients presented with neonatal seizures (range: 1–18 days) except for patient 24 who started with infantile spasms at age 6 months. Thirty (88%) patients had seizure onset within 3 days of life. At the onset, focal seizures with tonic component were the main seizure type, often associated with apnea/desaturation (14/34, 41%), but multiple seizure types, including tonic (16/34, 47%), clonic (6/34, 18%), tonic-clonic (9/34, 26%), and myoclonic (3/34, 9%) seizures, and spasms (5/34, 15%), were reported during the follow-up.

EEG data at onset were available for 31/34 patients and showed mainly burst suppression pattern (19/31, 61%) or multifocal epileptic activity (14/31). In 1 patient with late onset (24), hypsarrhythmia was reported. In 2 patients, interictal EEG was unremarkable. Interictal EEG at follow-up showed focal (10/34, 29%) or multifocal (12/34, 35%) epileptiform abnormalities or disorganized/slow background alone (11/34, 12%) and was unremarkable in 8 (24%) patients.

Brain MRI was unremarkable in 15 (44%) patients. Non-specific abnormalities (i.e., mild cerebral atrophy, hypoplasia of the corpus callosum, enlarged lateral ventricles, or delayed myelination) were detected in 16 (47%) subjects. At the onset, patients were on various drugs (table e-1, links.lww.com/NXG/A334). Phenobarbital was frequently used at the onset (24/34 individuals), but carbamazepine was the most used during the disease (18/34). At the last follow-up, 18 patients were on monotherapy (11 on carbamazepine), 11 on bitherapy (5 with carbamazepine), and 5 subjects were off-therapy.

Overall, 22 (65%) patients became seizure free within age 6 years (mean 1.2 years), and 12 (35%) still had seizures at a

Glossary

BFNE = benign neonatal familial epilepsy; DEE = developmental epileptic encephalopathy; ID = intellectual disability; TM = transmembrane; VSD = voltage sensor domain.
mean follow-up age of 3.8 years (range: 4 months-9 years). Three (9%) patients relapsed and showed rare focal seizures during childhood. Five patients (15%) showed unremarkable examination (mean follow-up age: 9.2 years), whereas axial hypotonia was reported in 22 (65%) subjects. At the last follow-up, 11 patients were able to walk independently and 3 with support; 21 patients were nonverbal or could pronounce only a few words/short sentences, and 5 had a normal speech. Cognitive outcome was variable, ranging from severe intellectual disability (ID) in 12, mild in 7, profound in 5, and moderate in 4 patients. Four individuals showed normal cognition at a mean follow-up age of 9.7 (range: 3.9–13) years.

Genetic findings
Overall, 28 de novo pathogenic variants were identified in 34 patients, mainly missense (25/28). Also, 2 splice site defects (c.1118+1G>A, c.927+5G>C) and 1 single amino acid deletion (c.910_912TTC, p.Phe305del) were detected. Seven variants are reported for the first time (c.560C>T, p.Ser187Phe; c.845A>T, p.Arg282Val; c.812G>A, p.Gly271Val; c.637C>T, p.Gly301Ser; c.901G>A, p.Gly301Ser) lie in the central cavity of the channel, 1 in the P-loop (Gly271) and 2 in the S6 helix (Ala294, Gly301) (figure). All but 1 (c.365C>T, p.Ser122Leu) TM variants are located in 2 distinct regions of the protein (figures e-1 and e-2, links.lww.com/NXG/A334).

Structural modeling
The Kv7.2 structural model allows us to pinpoint the position of all the mutated residues in the TM domain of the channel, including the 6 recurrent variants (figure). Three of them (i.e., c.587C>T, p.Arg198Gln; c.629G>A, p.Arg210His; and c.637C>T, p.Arg213Trp) are located in the VSD S4 helix. The c.587C>T, p.Arg198Gln variant is located toward the extracellular region, near the positively charged residue p.Arg198, which is itself involved in another variant of the cohort (c.593G>A, p.Arg198Tyr); the c.629G>A, p.Arg210His and the c.637C>T, p.Arg213Trp variants are in the inner part of the S4 domain. Finally, the other 3 variants (c.812G>A, p.Gly271Val; c.881C>T, p.Ala294Val; and c.901G>A, p.Gly301Ser) lie in the central cavity of the channel, 1 in the P-loop (Gly271) and 2 in the S6 helix (Ala294, Gly301) (figure). All but 1 (c.365C>T, p.Ser122Leu) TM variants are located in 2 distinct regions of the protein (figures e-1 and e-2, links.lww.com/NXG/A334).

Correlation between localization of missense variants and outcome
We organized the patients carrying missense variants into representative clusters to identify genotype-phenotype correlations (table e-2, links.lww.com/NXG/A334) by using 2 stratification models. Model 1 was based on the topological position of the variants: (1) patients with TM variants (n = 20, including the 6 recurrent changes); (2) patients with variants located in the C terminus and the different loops (n = 11). Model 2 was restricted to the patients carrying variants mapped...
Table 1 Genotype-phenotype correlations for the 6 recurrent variants

| Patient ID | 8 | 25 | 27 | 28 | 21 | 23 | 6 | 22 | 12 | 33 | 19 | 26 |
|------------|---|----|----|----|----|----|---|----|----|----|----|----|
| Onset (age) | 3 d | 2 d | 1 d | 2 d | 2 d | 1 d | 1 d | 15 d | 1 d | 1 d | 1 d | 2 d |
| Seizure at onset (frequency) | Focal tonic sz, clonic jerks, desaturation, and cyanosis (multiple) | Tonic-clonic sz (infrequent) | Tonic sz; focal sz (generalized sz (multiple) | Febrile tonic-clonic sz (sporadic) | Tonic sz with head deviation and cyanosis (multiple until 6 m) | Tonic sz, perioral cyanosis, laryngeal stridor, and autonomic features (multiple) | Focal tonic sz with clonic jerks and desaturation (multiple) | Eyelid mydriasis (multiple) | Tonic sz with head deviation, apnea, and bradycardia (multiple) | Focal motor sz with autonomic features (multiple or clusters) | Focal tonic asymmetric sz with head deviation, apnea, and cyanosis (multiple) |
| Seizure at follow-up | Tonic sz; gaze fixity, head deviation, clonic jerks; focal and generalized tonic-clonic sz, and severe desaturation | Tonic-clonic sz; tonic sz (sporadic) | Focal sz | Focal sz | Tonic sz with cyanosis, bradycardia, and head and eye deviation (febrile and atfebrile) wakefulness and in sleep | Clonic sz | None | Absence | Tonic-clonic sz (2 nocturnal episodes at 4 y 3 m) | Tonic sz and focal clonic sz with or without eyes deviation (daily and then sporadic) | None |
| Last evaluation | 4 m | 13 y | 7 y | 6 y 8 m | 3 y 1 m | 11 y | 1 y | 3 y 1 m | 12 y 1 m | 5 y | 4 y | 6 m |
| Current ASMs | PB and LEV | None | CBZ | CBZ | CBZ | VPA | TPM | None | CBZ | CBZ (at 4 y 3 m) | None | CBZ |
| EEG at onset | Slow background | Burst suppression, discontinuous background, and multifocal abnormalities | Burst suppression, discontinuous background, and multifocal abnormalities | Burst suppression, multifocal abnormalities, and discontinuous background | Burst suppression, multifocal abnormalities | Burst suppression, multifocal abnormalities, and discontinuous background | Burst suppression | Burst suppression pattern, discontinuous background, and multifocal abnormalities | Burst suppression, discontinuous background, and multifocal abnormalities | Burst suppression, discontinuous background, and multifocal abnormalities | Burst suppression, discontinuous background, and multifocal abnormalities |
| Last EEG (age) | Focal bilateral P abnormalities in sleep (4 m) | Focal abnormalities (13 y) | Unremarkable (7 y) | Focal abnormalities (6 y 8 m) | Unremarkable (3 y 1 m) | Multifocal abnormalities and poor background (11 y 8 m) | Multifocal abnormalities and disorganized (1 y) | Unremarkable (3 y 1 m) | Poor background and no epileptic abnormalities (12 y) | Multifocal epileptic activity (4 y 11 m) | Focal spike-wave in F areas in sleep (4 y) | Focal epileptic activity, spike-slow wave over O areas (12 m) |
| ID (age) | Not applicable (4 m) | Normal (13 y) | Mild (5 y) | Moderate (6 y 8 m) | Mild (3 y) | Moderate (11 y) | Profound (1 y) | Mild (3 y) | Severe (11 y 8 m) | Profound (4 y) | Mild (4 y) | Mild (1 y) |
| Neurologic examination | Mild global hypotonia, good eye contact, and good head control | Normal, good head control, independent walking, and normal speech | Normal, good head control, independent walking, and normal speech | Normotonic, independent walking with clumsiness, macrocephaly, and ASD with language | Hypotonia, good head control, poor eye contact, delayed walking, and poor speech | Ataxic, assisted walking, poor speech, and learning disorders | Severe hypotonia, poor eye contact, no head control, no walking, and no speech | Good head control, assisted walking with clumsiness, and poor speech (language disorder) | Hypotonia, no walking, and poor speech | Axial hypotonia, dystonic quadriaparesis, delayed head control, no eye contact, no speech, and no walking | Mild global hypotonia, good head control, good eye contact, delayed independent walking, and poor speech | Mild axial hypotonia, good head control, good eye contact, no walking, and no speech |

Abbreviations: ASMs = antiseizure medications; CBZ = carbamazepine; F = frontal; ID = intellectual disability; LEV = levetiracetam; m = month(s); O = occipital; P = parietal; PB = phenobarbital; sz = seizure; T = temporal; TPM = topiramate; VPA = valproate; y = year(s).
by the 3D model, which includes variants localized at VSD (n = 10, in the S3-S4 linker and S4 helix) and pore cavity (n = 13). Both models (table e-3, links.lww.com/NXG/A334) failed to show a correlation between localization of the variants (TM vs others) and patients’ cognitive outcome (normal/abnormal), whereas analysis of time to seizure offset (≤1 year/>1 year) showed a trend toward significance for model 2 (p = 0.08).

Genotype-phenotype correlations for the 6 recurrent variants are shown in table 1. One patient was still too young to evaluate development (ID 8). All other individuals showed some degree of cognitive impairment. Nevertheless, there was no correspondence between seizure offset and cognitive outcome for patients carrying 3 variants, i.e., c.587C>T, p.Ala196Val (ID #8, #25), c.629G>A, p.Arg210His (ID #27, #28); and c.812G>A, p.Gly271Asp (ID #6, #22). Likewise, patients carrying the other recurrent 3 variants displayed similar cognitive outcome despite quite different electroclinical features and epilepsy duration.

Discussion

We report 34 patients with epilepsy with de novo KCNQ2 variants, including 7 with novel pathogenic changes. All but 1 patient (24) presented in the neonatal period with focal seizures with predominant tonic component followed by autonomic features and clonic jerks. Interictal EEG at onset varied from burst suppression pattern to multifocal epileptiform abnormalities or normal background. One patient (24) with c.593G>A, p.Arg198Gln variant presented at age 6 months with clusters of epileptic spasms and hypsarrhythmia, confirming that this specific mutation, leading to a gain-of-function effect, is associated with West syndrome without neonatal seizures.10

Our cohort showed a wide phenotypic spectrum ranging from an age-dependent, self-limiting epilepsy with normal cognitive development to a severe DEE, but also an intermediate phenotype featuring neonatal epilepsy or DEE.11,18 Moreover, up to 13% of the subjects with a de novo KCNQ2 variant showed normal developmental outcome and a clinical course consistent with self-limiting neonatal epilepsy.

Most MRI examinations performed at onset and during follow-up were normal or showed nonspecific abnormalities. Carbamazepine was the most used drug during the disease, alone or in combination, confirming its effectiveness in these patients.5,13

Most patients harbored missense pathogenic variants, which clustered in conserved regions of the protein (S4 helix, pore loop, and S6 helix), consistent with previous reports.14,15 More than half of the variants associated with severe or profound ID were localized in the pore region, according to previous studies.16,17

Although the correlation analysis between localization of variants and time to seizure offset shows a trend toward significance for model 2, genotype-phenotype correlations were elusive in our cohort, confirming the complexity of variant interpretation to assess the impact of the single mutation. These findings are only in part surprising. In fact, in several genetic epilepsies, pathogenic variants of the same gene may result in different and contrasting epilepsy phenotypes, causing, for example, either self-limiting epilepsy or DEE.11,18

It is widely accepted that cognitive dysfunction in epilepsy is related to multiple factors, such as therapy, seizure frequency/severity, and, not lastly, the possible role of gene modifiers and nongenetic factors, as described for other genetic DEEs.18 Accordingly, in our cohort, patients with recurrent variants showed the same age at onset but not exactly overlapping electroclinical features, treatment response, or cognitive outcome. Nevertheless, specific missense de novo KCNQ2 variants (R201C and R201H) consistently present with a very severe form of neonatal encephalopathy.19

Our study has several limitations. First, some group numbers are very low, potentially leading to a lack of statistical power. Second, the effect of the pathogenic variants on motor, language, and social skills could be only indirectly inferred in our patients due to the retrospective nature of the study. Third, we used homology-based structural modeling because no experimentally determined Kv7.2 structure is available, but we did not associate any pathogenic score (e.g., PROVEAN, Protein Variation Effect Analyzer) or specific algorithm to predict the impact of the pathogenic variants.16 The added value to genotype-phenotype correlation of tridimensional structural modeling deserves further studies.

In conclusion, this study highlights the complexity of variant interpretation to assess the impact of de novo KCNQ2 mutations, especially on neurocognitive outcome beyond the early and often transient epilepsy. Genetic modifiers could be implicated, but the study paradigms to successfully address this issue need to be developed.

Study funding

This work was developed within the framework of the DINOGMI Department of Excellence of MIUR 2018–2022 (legge 232 del 2016).

Disclosure

A. Coppola has received a speaker fee for Eisai. S. Weckhuysen has received speaker and consultancy fees from Biodex, Zogenix, UCB, Xenon, and Lundbeck. P. Striano has received speaker fees and participated at advisory boards for Biogen, Zogenix, and GW Pharmaceuticals and has received research funding by ENECTA BV, GW Pharmaceuticals, Kolfarma srl., and Eisai. The other authors do not report any disclosure. Go to Neurology.org/NG for full disclosures.

Publication history

Received by Neurology: Genetics August 5, 2020. Accepted in final form October 6, 2020.
### Appendix Authors

| Name                      | Location                                                                 | Contribution                                                                 |
|---------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Federica Malerba, MD      | Universitá degli Studi di Genova, Italy, IRCCS Istituto G. Gaslini, Genova, Italy | Analyzed the data, wrote the manuscript, interpreted the data, and revised the manuscript |
| Giulio Alberini, PhD      | Istituto Italiano di Tecnologia, Genova, Italy                            | Analyzed the data, wrote the manuscript, interpreted the data, and revised the manuscript |
| Ganna Balagura, MD        | Universitá degli Studi di Genova, Italy, IRCCS Istituto G. Gaslini, Genova, Italy | Analyzed the data, interpreted the data, and revised the manuscript            |
| Francesca Marchese, MD    | IRCCS Istituto G. Gaslini, Genova, Italy                                  | Analyzed the data, major role in the acquisition of data, and revised the manuscript |
| Elisabetta Amadori, MD    | Universitá degli Studi di Genova, Italy, IRCCS Istituto G. Gaslini, Genova, Italy | Revised the manuscript                                                        |
| Antonella Riva, MD        | Universitá degli Studi di Genova, Italy, IRCCS Istituto G. Gaslini, Genova, Italy | Revised the manuscript                                                        |
| Maria Stella Vari, MD, PhD| Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto G. Gaslini, Genova, Italy | Analyzed the data, major role in the acquisition of data, interpreted the data, and revised the manuscript |
| Elena Gennaro, PhD        | IRCCS Istituto G. Gaslini, Genova, Italy                                  | Major role in the acquisition of data and revised the manuscript              |
| Francesca Madia, PhD      | IRCCS Istituto G. Gaslini, Genova, Italy                                  | Major role in the acquisition of data and revised the manuscript              |
| Vincenzo Salpietro, MD, PhD| Universitá degli Studi di Genova, Italy, IRCCS Istituto G. Gaslini, Genova, Italy | Wrote the manuscript and revised the manuscript                              |
| Marco Angriman, MD        | Central Hospital of Bolzano, Bolzano, Italy                               | Major role in the acquisition of data and revised the manuscript              |
| Lucio Giordano, MD        | ASST Spedali Civili, Brescia, Italy                                       | Major role in the acquisition of data and revised the manuscript              |
| Patrizia Accorsi, MD      | ASST Spedali Civili, Brescia, Italy                                       | Major role in the acquisition of data and revised the manuscript              |
| Marina Trivisano, MD      | Bambino Gesù Children's Hospital, IRCCS, Roma, Italy                      | Major role in the acquisition of data and revised the manuscript              |
| Nicola Specchio, MD       | Bambino Gesù Children's Hospital, IRCCS, Roma, Italy                      | Major role in the acquisition of data and revised the manuscript              |
| Angelo Russo, MD          | IRCCS Institute of Neurological Sciences of Bologna, Italy                | Major role in the acquisition of data and revised the manuscript              |
| Giuseppe Gobbi, MD        | IRCCS, Institute of Neurological Sciences of Bologna, Bologna, Italy       | Major role in the acquisition of data and revised the manuscript              |

### Appendix (continued)

| Name                      | Location                                                                 | Contribution                                                                 |
|---------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Federico Raviglione, MD   | U.O.N.P.I.A. ASST-Rhodense, Rho, Milano, Italy                            | Major role in the acquisition of data and revised the manuscript              |
| Tiziana Pisano, MD        | A. Meyer Children's Hospital, Firenze, Italy                              | Major role in the acquisition of data and revised the manuscript              |
| Carla Marini, MD, PhD     | Pediatric Hospital G. Salesi, United Hospital of Ancona, Italy             | Analyzed the data, major role in the acquisition of data, wrote the manuscript, interpreted the data, and revised the manuscript |
| Maria M. Mancardi, MD     | IRCCS Istituto G. Gaslini, Genova, Italy                                  | Major role in the acquisition of data and revised the manuscript              |
| Lino Nobili, MD, PhD      | Universitá degli Studi di Genova, Italy, IRCCS Istituto G. Gaslini, Genova, Italy | Major role in the acquisition of data and revised the manuscript              |
| Elena Freri, MD           | Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy          | Major role in the acquisition of data and revised the manuscript              |
| Barbara Castellotti, MD   | Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy          | Major role in the acquisition of data and revised the manuscript              |
| Giuseppe Capovilla, MD    | Epilepsy Center, C. Poma Hospital, Mantova, Italy, Fondazione Poliambulanza Brescia, Italy | Major role in the acquisition of data and revised the manuscript |
| Antonietta Coppola, MD    | Universitá degli Studi di Napoli Federico II, Napoli, Italy               | Major role in the acquisition of data and revised the manuscript              |
| Alberto Verrotti, MD      | University of Perugia, Perugia, Italy                                     | Revised the manuscript                                                        |
| Paola Martelli, MD        | ASST Spedali Civili, Brescia, Italy                                       | Major role in the acquisition of data                                         |
| Francesco Miceli, PhD     | Universitá degli Studi di Napoli Federico II, Napoli, Italy               | Revised the manuscript                                                        |
| Luca Maraglino, PhD       | Istituto Italiano di Tecnologia, Genova, Italy, IRCCS Ospedale Policlinico San Martino, Genova, Italy | Wrote the manuscript and revised the manuscript                              |
| Fabio Benfenati, MD, PhD  | Istituto Italiano di Tecnologia, Genova, Italy, IRCCS Ospedale Policlinico San Martino, Genova, Italy | Wrote the manuscript and revised the manuscript                              |
| Maria R. Cilio, MD        | Saint-Luc University Hospital, and Institute of Experimental and Clinical Research (IREC), Université Catholique de Louvain, Brussels, Belgium | Analyzed the data, wrote the manuscript, interpreted the data, and revised the manuscript |
| Kathrine M. Johannesen, MD| The Danish Epilepsy Center Filadelfia, Dianalund, Denmark Institute for Regional Health Services, University of Southern Denmark, Odense, Denmark | Major role in the acquisition of data and revised the manuscript |
Appendix (continued)

| Name                  | Location                                                                 | Contribution                                                                 |
|-----------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Rikke S. Møller, PhD  | The Danish Epilepsy Center, Filadelfia, Dianalund, Denmark                | Analyzed the data, major role in the acquisition of data, wrote the manuscript, interpreted the data, and revised the manuscript |
|                       | University of Southern, Odense, Denmark                                    |                                                                              |
| Berten Ceulemans, MD  | University Hospital Antwerp, Antwerp, Belgium                              | Major role in the acquisition of data and revised the manuscript              |
| Carlo Minetti, MD, PhD | Università degli Studi di Genova, Italy                                   | Revised the manuscript                                                       |
|                       | IRCCS Istituto G. Gaslini, Genova, Italy                                   |                                                                              |
| Sarah Weckhuysen, MD, PhD | University Hospital Antwerp, Antwerp, Belgium, Belgium                      | Analyzed the data, major role in the acquisition of data, wrote the manuscript, interpreted the data, and revised the manuscript |
|                       | Molecular Neurology, Antwerp, University Hospital, University of Antwerp, Belgium |                                                                              |
| Federico Zara, PhD    | Università degli Studi di Genova, Italy                                    | Analyzed the data, wrote the manuscript, interpreted the data, and revised the manuscript |
|                       | IRCCS Istituto G. Gaslini, Genova, Italy                                   |                                                                              |
| Maurizio Taglialatela, PhD | Università degli Studi di Napoli Federico II, Napoli, Italy             | Analyzed the data, wrote the manuscript, interpreted the data, and revised the manuscript |
|                       | Italy                                                                      |                                                                              |
| Pasquale Striano, MD, PhD | Università degli Studi di Genova, Italy                                   | Designed and conceptualized the study, analyzed the data, major role in the acquisition of data, wrote the manuscript, interpreted the data, and revised the manuscript |
|                       | IRCCS Istituto G. Gaslini, Genova, Italy                                   |                                                                              |

References

1. Miceli F, Soldovieri MV, Joshi N, et al. KCNQ2-related disorders. In: Adam MP, Ardinger HH, Pagon RA, et al. editors. GeneReviews® [Internet]. Seattle: University of Washington; 2010. Available at: ncbi.nlm.nih.gov/books/NBK3234/; 2018.
2. Orhan G, Bock M, Schepers D, et al. Dominant-negative effects of KCNQ2 mutations are associated with epileptic encephalopathy. Ann Neurol 2014;75:382–394.
3. Millichap JJ, Park KL, Tsuchida T, et al. KCNQ2 encephalopathy: features, mutational hot spots, and ezogabine treatment of 11 patients. Neurol Genet 2016;2:e96.
4. Miceli F, Soldovieri MV, Ambrosio P, et al. Genotype–phenotype correlations in neonatal epilepsies caused by mutations in the voltage sensor of Kv7.2 potassium channel subunits. Proc Natl Acad Sci USA 2013;110:4386–4391.
5. Numis AL, Angirman M, Sullivan JE, et al. KCNQ2 encephalopathy: delineation of the electroclinical phenotype and treatment response. Neurology 2014;82:368–370.
6. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424.
7. Ashkenazy H, Erez E, Marts E, Pupko T, Ben-Tal N. Consurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucleic Acids Res 2010;38:W529–W533.
8. Zhou X, Ma A, Liu X, et al. Infantile seizures and other epileptic phenotypes in a Chinese family with a missense mutation of KCNQ2. Eur J Pediatr 2006;165:691–695.
9. Mihl M, Boutry-Kryza N, Sutera-Sardo J, et al. Similar early characteristics but variable neurological outcome of patients with a de novo mutation of KCNQ2. Orphanet J Rare Dis 2013;8:80.
10. Millichap JJ, Miceli F, De Maria M, et al. Infantile spasms and encephalopathy without preceding neonatal seizures caused by KCNQ2 R198Q, a gain-of-function variant. Epilepsia 2017;58:e10–e15.
11. Johannesen K, Marinu C, Pfeffer S, et al. Phenotypic spectrum of GABRA1: from generalized epilepsies to severe epileptic encephalopathies. Neurology 2016;87:1140–1151.
12. Johannesen KM, Gardella E, Encinas AC, et al. The spectrum of intermediate SCN8A-related epilepsy. Epilepsia 2019;60:830–844.
13. Pisano T, Numis AL, Heavin SB, et al. Early and effective treatment of KCNQ2 encephalopathy. Epilepsia 2015;56:685–691.
14. Kato M, Yamagata T, Kihata M, et al. Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation. Epilepsia 2013;54:1282–1287.
15. Weckhuysen S, Mandelstam S, Suls A, et al. KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. Ann Neurol 2012;71:15–25.
16. Goto A, Ishii A, Shibata M, Ibara Y, Cooper EC, Hirose S. Characteristics of KCNQ2 variants causing either benign neonatal epilepsy or developmental and epileptic encephalopathies. Epilepsia 2019;60:1870–1880.
17. Zhang J, Kim EC, Chen C, et al. Identifying mutation hotspots reveal pathogenetic mechanisms of KCNQ2 epileptic encephalopathy. Sci Rep 2020;10:4756.
18. Heiling I, Tayoun AA. Understanding genotypes and phenotypes in epileptic encephalopathies. Mol Syndromol 2016;7:172–181.
19. Mulkey SB, Ben-Zerev B, Nicolai J, et al. Neonatal nonepileptic myoclonus is a prominent clinical feature of KCNQ2 gain-of-function variants R201C and R201H. Epilepsia 2017;58:436–445.