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

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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.

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Glossary

BFNE = benign neonatal familial epilepsy; **DEE** = developmental epileptic encephalopathy; **ID** = intellectual disability; **TM** = transmembrane; **VSD** = voltage sensor domain.

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 classification[6] 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

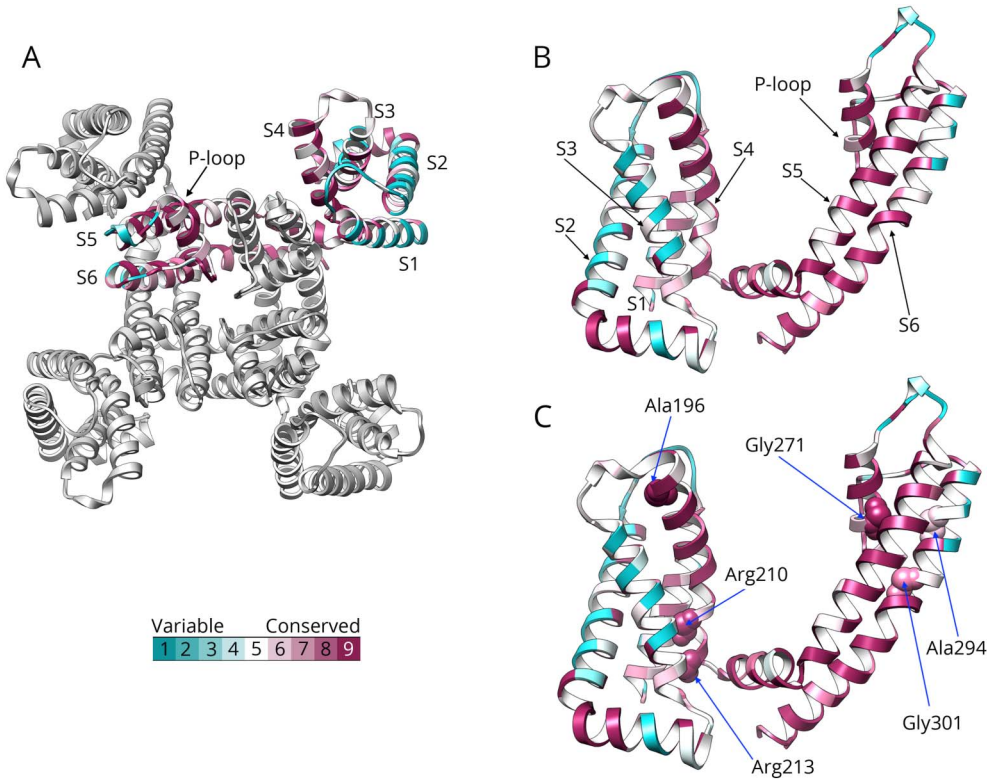
Thirty-four patients (23 females) were included (table e-1, links.lww.com/NXG/A334). Age at the last follow-up ranged from 4 months to 13 years (mean: 4.7 ± 3.7 years). All patients presented with neonatal seizures (range: 1–18 days) except for patient 24 who started with infantile spasms at age 6 months. 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. Nonspecific 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

Figure Structural modeling of the Kv7.2 channel



(A) Extracellular view of the representative of the homotetrameric Kv7.2 channel model in ribbon style. One of the subunits is colored by conservation grades according to the ConSurf software (highly conserved residues are shown in maroon, average ones in white, and highly variable ones in turquoise). Secondary structural elements are labeled. (B) Lateral view of 1 channel subunit. (C) Visualization of the 6 residues involved in the mutations occurring twice in the cohort. Backbone atoms are represented as spheres.

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.Asp282Val; c.812G>A, p.Gly271Asp; c.569A>T, p.Gly189Ile; c.1665C>G, p.Phe555Leu; c.1642G>C, and p.Asp548His; c.927+5G>C), whereas the others are reported in RIKEE (rikee.org) and ClinVar (www.ncbi.nlm.nih.gov/clinvar/) databases. Two of the mutated residues of the unpublished pool are involved in other variants reported by the literature, such as c.812G>T, p.Gly271Val,[8] and c.566G>T, p.Gly189Val.[9]

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.Ala196Val; c.629G>A, p.Arg210His; and c.637C>T, p.Arg213Trp) are located in the VSD S4 helix. The c.587C>T, p.Ala196Val 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.Arg198Gln); 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.Gly271Asp; 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

	c.587C>T, p.Ala196Val		c.629G>A, p.Arg210His		c.637C>T, p.Arg213Trp		c.812G>A, p.Gly271Asp		c.881C>T, p.Ala294Val		c.901G>A, p.Gly301Ser	
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 jerkings, desaturation, and cyanosis (multiple)	Tonic-clonic sz (infrequent)	Tonic sz; focal sz (multiple)	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 jerkings and desaturation (multiple)	Eyelid myoclonia (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, gaxe's fixity, head deviation, clonic jerkings; focal and generalized tonic-clonic sz, and severe desaturation	Tonic-clonic sz; tonic sz (sporadic)	Focal sz	Focal sz	Febrile tonic-clonic sz (sporadic)	Tonic sz with cyanosis, bradycardia, and head and eyes deviation (febrile and afebrile) 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
Seizure offset (age)	No	Yes (4 m). Relapsed (30 m, 4 y, 12 y)	Yes (4 y)	No	Yes (2.5 y)	Yes (4 y)	Yes (4 m)	Yes (1 y)	Yes (6 y)	Yes (3 m–4 y 3 m). Relapsed (4 y 3 m)	Yes (6 m)	Yes (24 d)
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	Burst suppression	Unremarkable	Multifocal abnormalities	Burst suppression, multifocal abnormalities, and discontinuous background	Multifocal abnormalities	NA	Burst suppression	Burst suppression pattern, discontinuous background, and multifocal abnormalities	Burst suppression, multifocal abnormalities, and discontinuous background
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	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 quadriparesis, 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 in terms of intellectual outcomes and time to reach seizure freedom, as described for other disorders.[11,12] Noteworthy, an intermediate phenotype featuring neonatal epilepsy with mild/moderate ID occurred in a third of patients. 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.

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Disclosure

A. Coppola has received a speaker fee for Eisai. S. Weckhuysen has received speaker and consultancy fees from Biocodex, Zogenix, UCB, Xenon, and Lundbeck. P. Striano has received speaker fees and participated at advisory boards for Biomarin, 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.

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Kathrine M. Johannesen, MD	The Danish Epilepsy Center Filadelfia, Dianalund, DenmarkInstitute 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, DenmarkInstitute for Regional Health Services, University of Southern Denmark, Odense, Denmark	Analyzed the data, major role in the acquisition of data, wrote the manuscript, interpreted the data, and revised the manuscript
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 IRCCS Istituto G. Gaslini, Genova, Italy	Revised the manuscript
Sarah Weckhuysen, MD, PhD	University Hospital Antwerp, Antwerp, BelgiumVIB-Center for Molecular Neurology, Antwerp, Belgium Institute Born-Bunge, University of Antwerp, Belgium	Analyzed the data, major role in the acquisition of data, wrote the manuscript, interpreted the data, and revised the manuscript
Federico Zara, PhD	Università degli Studi di Genova, Italy IRCCS Istituto G. Gaslini, Genova, Italy	Analyzed the data, wrote the manuscript, interpreted the data, and revised the manuscript
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
Pasquale Striano, MD, PhD	Università degli Studi di Genova, Italy IRCCS Istituto G. Gaslini, 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

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