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# Distinctive amplitude-integrated EEG ictal pattern and targeted therapy with carbamazepine in KCNQ2 and KCNQ3 neonatal epilepsy: a case series

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Abstract:**
Background: Carbamazepine (CBZ) is effective in treating KCNQ2/3 related seizures, which may present with a distinctive aEEG pattern.
Objective: To assess how improved recognition of the distinctive aEEG ictal pattern associated with KCNQ2/3 variants has enabled early and effective targeted therapy with CBZ.
Methods: Retrospective descriptive study of 5 neonates with KCNQ2/3 pathogenic gene variants admitted at a level three neonatal intensive care unit (NICU) over an eight-year period.
Results: The distinctive ictal aEEG pattern was recognized in 4 neonates after an average of 61.5 hours (minimum 12 h, maximum 120h) from the first electroclinical seizure and prompted the use of CBZ that was effective in all. The two most recently diagnosed patients could avoid polytherapy as they received CBZ as the 1st and 2nd antiseizure medication (ASM) respectively. Three out of five patients with continuous normal voltage (CNV), sleep wake cycling (SWC) and shorter post-ictal suppression had normal neurodevelopmental outcome. Regarding the remaining two infants, one was not trialled with CBZ and had a high seizure burden, both presented with a prolonged post-ictal suppression, no SWC and had moderate-to-severe developmental delay. Genetic results became available after the neonatal period in all but one of the infants, who had a prenatal diagnosis.
Conclusions: Recognition of the distinctive ictal aEEG pattern in the NICU allowed early and effective targeted therapy with CBZ in four neonates, well before genetic results became available. Furthermore, a CNV background pattern with SWC and short post-ictal suppression were associated with normal developmental outcomes.

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# Distinctive amplitude-integrated EEG ictal pattern and targeted therapy with carbamazepine in KCNQ2 and KCNQ3 neonatal epilepsy: a case series

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Abstract

**Background:** Carbamazepine (CBZ) is effective in treating KCNQ2/3 related seizures, which may present with a distinctive aEEG pattern.

**Objective:** To assess how improved recognition of the distinctive aEEG ictal pattern associated with *KCNQ2/3* variants has enabled early and effective targeted therapy with CBZ.

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**Methods:** Retrospective descriptive study of 5 neonates with *KCNQ2/3* pathogenic gene variants admitted at a level three neonatal intensive care unit (NICU) over an eight-year period.

**Results:** The distinctive ictal aEEG pattern was recognized in 4 neonates after an average of 61.5 hours (minimum 12 h, maximum 120h) from the first electroclinical seizure and prompted the use of CBZ that was effective in all. The two most recently diagnosed patients could avoid polytherapy as they received CBZ as the 1$^{st}$ and 2$^{nd}$ antiseizure medication (ASM) respectively. Three out of five patients with continuous normal voltage (CNV), sleep wake cycling (SWC) and shorter post-ictal suppression had normal neurodevelopmental outcome. Regarding the remaining two infants, one was not trialled with CBZ and had a high seizure burden, both presented with a prolonged post-ictal suppression, no SWC and had moderate-to-severe developmental delay. Genetic results became available after the neonatal period in all but one of the infants, who had a prenatal diagnosis.

**Conclusions:** Recognition of the distinctive ictal aEEG pattern in the NICU allowed early and effective targeted therapy with CBZ in four neonates, well before genetic results became available. Furthermore, a CNV background pattern with SWC and short post-ictal suppression were associated with normal developmental outcomes.

Keywords

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*KCNQ2*, *KCNQ3*, neonatal seizures, amplitude-integrated EEG, carbamazepine, post-ictal suppression

## Introduction

Diagnosing and managing neonatal seizures can be challenging. While most seizures occur in response to a brain insult, in 13-15% of infants it represents the onset of epilepsy [1 2]. Pathogenic variants in *KCNQ2* and *KCNQ3* genes, encoding for voltage-gated potassium channel subunits underlying the neuronal M-current (IKM), are among the most common genetic causes of early onset epilepsy, with phenotypes of varying severity [1 3 4]. Self-limited neonatal epilepsy (SLNE) occurs mostly in an autosomal dominant inheritance and is typically associated with a good outcome, both regarding seizures and development, although a correlation between seizure frequency during the neonatal period and epilepsy later in life was estimated in up to 25% [1 5]. In contrast, genetic pathogenic variants arising *de novo* are responsible for over 10% of developmental and epileptic encephalopathy (DEE), the more severe end of the spectrum [4]. Despite this heterogeneity in neurological outcome, seizure semiology is similar in KCNQ2-SLNE and DEE, and is characterized by a sequence of features initiated by a tonic phase with asymmetric posturing most often accompanied by apnea and desaturation followed by unilateral or bilateral clonic jerks [1 2 6]. Seizures in DEE can start as early as the first day of life while infants with SLNE usually start on day 3-5 of life. Seizures are brief but frequent, occurring up to 30 times/day or even evolving to status epilepticus (SE) in SLNE and up to 35 seizures per hour in DEE [1 4]. Seizures are usually refractory to the most common first line antiseizure

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medication (ASM), such as phenobarbital (PB), and are typically controlled by sodium channel-blockers (SCB) including carbamazepine (CBZ)/oxcarbazepine (OXC), phenytoin and lidocaine [1 6-8]. As these genetic pathogenic variants result in functional rather than structural anomalies, the brain magnetic resonance imaging (MRI) is non-specific, ranging from normal to showing a thin corpus callosum, diffuse hypomyelination or hyperintensities in the basal ganglia and thalamus that later resolve [4]. Although conventional video-EEGs (vEEGs) are considered the gold standard for seizure diagnosis, amplitude-integrated electroencephalography (aEEG) has proven to be useful for seizure detection, especially with access to the raw EEG, and is used routinely in an increasing number of neonatal intensive care units (NICU) [2 9]. The most common appearance of an aEEG single seizure is a transient rise in the amplitude lower and upper margin [10]. In *KCNQ2*-related epilepsies, a marked and prolonged suppression of the amplitude at the end of the seizure has been recognized [10-12]. The ictal aEEG feature can be more easily seen in less time compressed recordings at 15cm/h, as opposed to the routine 6cm/h [10 11]. This distinctive ictal aEEG pattern has been described in both SLNE and DEE associated *KCNQ2* pathogenic variants. Early recognition of the electroclinical phenotype at the bedside by using aEEG may direct genetic testing and allow early use of SCB [11]. Since the first report in 2017, several studies have highlighted this distinctive electroclinical phenotype and targeted treatment with SCB [13-16]. More recently, the same electroclinical phenotype was observed in *SCN2A* and *KCNQ3*-related epilepsies [17]. Interestingly, infants with SCN2A- and KCNQ3-epilepsies share with KCNQ2-related epilepsies the tonic seizure semiology, suggesting that the typical electrographic pattern may be mechanistically related to the pronounced tonic seizure element [17].

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Because genetic epilepsy is uncommon and not always easy to diagnose, we hereby present a single center case series of *KCNQ2/3*-related epilepsy, diagnosed after the first observation in 2017 and discuss the role of the aEEG/cEEG not only in the recognition of the ictal pattern and its usefulness in targeting therapy with SCB but also in providing early biomarkers that could be predictive of neurodevelopmental outcome.

**Methods:**

This is a retrospective, descriptive case series of 5 Infants with *KCNQ2/3* pathogenic gene variants, admitted between 2014 and 2022 to the NICU of the Centro Hospitalar Universitário São João (CHUSJ). According to our seizure management protocol, all newborns admitted in our NICU because of clinical seizures or because of high risk of developing seizures had aEEG/cEEG monitoring soon after admission. Patients who presented with a distinctive aEEG ictal pattern were consecutively included.

Seizure semiology was classified according to the International League Against Epilepsy (ILAE) classification as clonic, tonic, sequential, myoclonic and electrographic only [2].

A stepwise diagnostic workup was performed in all infants but one, to clarify the etiology including a detailed family history, investigation for infections and metabolic disorders, brain imaging and genetic testing.

*aEEG/cEEG monitoring*

As our standard protocol, a two-channel (C3-P3; C4-P4) video-aEEG machine (NicoletOne, Natus, Seatle, WA, USA) with simultaneous display of the raw EEG trace was performed in all patients soon after admission. As soon as possible, a full montage video-EEG was performed using the same machine , according to the international 10-

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20 system for electrode placement modified for neonates [18] with dual channel aEEG (aEEG/cEEG). Continuous brain monitoring was continued for at least 24 hours after the last electrographic seizure.

The aEEG was analyzed for the presence of the distinctive aEEG pattern consisting of a sudden rise of the lower and upper margin followed by a marked amplitude suppression [10 11]. The raw EEG was simultaneously analyzed. The aEEG was displayed at the bedside for real time evaluation by the attending neonatologist, and then reviewed by an experienced reader (AV, JMR) for the purpose of the study.

The aEEG tracing was divided into 6 hours sections. Each section was analyzed for background pattern, presence of sleep wake cycle (SWC) and seizures. Background pattern was classified using pattern recognition into continuous normal voltage (CNV), discontinuous normal voltage (DNV), continuous low voltage (CLV), burst suppression (BS) and flat trace (FT) [10]. The frequency of electroclinical seizures were classified as single seizure, repetitive seizures (3 or more seizures during a 30-minute period but not SE), and SE (continuously ongoing seizure activity for > 30 minutes or recurrent seizures for greater than or equal to 50% of the recording time) [19]. The post-ictal amplitude suppression was defined, according to Lhatoo et al [20] as the time interval between the immediate cessation of the ictal pattern and the appearance of burst suppression or continuous slowing, with suppression below 10 μV. The post-ictal amplitude suppression of the 1$^{st}$ electroclinical seizure was measured. The mean duration of the post-ictal suppression of the following seizures prior to start of CBZ was measured. The time interval from the first electroclinical seizure to the recognition of the typical pattern and administration of CBZ was calculated.. ASM was marked in the aEEG/cEEG whenever administered to the neonates and response was classified as

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effective (neonates no longer had seizures after the initiation of therapy and maintained seizure free till discharge), partial effective (reduction in frequency or 24 hours free of seizures) and non-responders (seizures persisted during hospitalization). ASM effect on electrocortical background activity was observed.

*Genetic investigations*

Genetic testing comprised *KCNQ2* next generation sequencing (NGS) (patient 1 and 2), epilepsy multigene NGS panel (patient 3 and 4) and testing for familial variants by Sanger sequencing (patient 5). Segregation studies were performed on parental samples for all patients. The following mRNA transcripts were used for variants annotation: *KCNQ2* (NM_172107.2), *KCNQ3* (NM_004519.4). Variants were classified according to the standards and guidelines provided by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology [21].

*Neurodevelopmental outcome*

Development, neuromotor and cognition were assessed using developmental milestones and neurological examination at discharge and during follow-up.

The study was approved by the ethics committee of CHUSJ and parental informed consent was obtained for all cases.

**Results**

Five infants were included. Clinical, aEEG, ASM and neuroradiological features are summarized in table 1.

*Clinical features*

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All infants were born at term, with clinical manifestations within the first 48 hours after birth (median 30h, range 20h-48h). Upon admission, patients 1 and 4 had an abnormal neurological examination, with hypoactivity, no gaze fixation. Patients 1, 2 and 4 required tube feeding.

Seizures were brief, with a frequency ranging from 3 per day to 6-9 per day. All patients had seizures characterized by an initial tonic phase with autonomic features. In 4 patients the tonic phase was followed by unilateral clonic jerks. Infections and inborn errors of metabolism workup was performed in all infants except patient 5 and was negative.

All infants had an MRI in the first week of life, except patient 5 who only had cranial ultrasound (cUS) that was normal.

Family history for seizures was positive in patient 5.

*aEEG/cEEG findings and ASM*

Patient 1 had received a loading dose of PB (10mg/kg iv) at 36 hours of life and then an additional 10 mg/kg iv. A trial of pyrodixine was attempted with no response. At our unit while on aEEG/cEEG monitoring, he presented with repetitive seizures unresponsive to levetiracetam and to a trial of intravenous pyridoxine followed by oral pyridoxal-5-phosphate and evolved to SE. Midazolam infusion was ineffective but lidocaine was able to control the SE and was effective during the 30 hrs of intravenous administration (loading dose of 2mg/kg in 10 min, followed by a continuous infusion of 6 mg/kg per hour for 6 hours, then 4 mg/kg per hour for 12 hours, followed by 2 mg/kg per hour for 12 hours), but seizures recurred once it was stopped. A combination of clonazepam, topiramate and valproic acid (VPA) allowed for seizure control at 30 days of life. Patient 2 presented with repetitive seizures with no response to a loading dose

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of PB (20 mg/kg iv) and a partial response to levetiracetam. A trial of pyridoxine was also attempted but seizures recurred and CBZ was used and noted to be effective. Patient 3 had a loading dose of PB at 48 hours of life (10 mg/kg iv) and was admitted to our unit at 72 hours of life. He had single seizures, as patient 5 and CBZ was the 2$^{nd}$ and the 1$^{st}$ASM respectively. Patient 4 had a loading dose of PB (10 mg/kg iv) at 20 hours of life and then an additional 10 mg/kg iv. He was transferred at 72 hours of life to our unit with one single electroclinical seizure in the 1$^{st}$ 24h of monitoring with a partial response to a loading dose of levetiracetam. Later seizures reappeared and became repetitive. Administration of CBZ resulted in seizure freedom.

All patients, except patient 1, were seizure free by the end of the first week of life. The median time of aEEG/cEEG recording was 7 days (range 2- 15 days).

The ictal recording showed the distinctive aEEG pattern in all patients by a sudden rise of the lower and upper margin followed by amplitude suppression. Interictally 4 patients had a CNV background and 1 a DNV. At 15 cm/h the ictal pattern and the post-ictal suppression was more easily recognized (Figure A). Post-ictal amplitude suppression of the first electroclinical seizure ranged between 52-192 seconds. The longest duration of suppression (192 sec) was observed in the infant with a DNV pattern. SWC was present in 3 patients. Patients with CNV had bilateral and unilateral ictal patterns (Figure B).

*Genetic findings*

In patient 1, *KCNQ2* gene sequencing was performed at 3 months of age, while in patients 2, 3 and 4, genetic testing was performed immediately after distinctive aEEG pattern was recognized with results available at 2, 5 and 3 months of age respectively.

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In patient 5 a prenatal molecular diagnosis was performed by familial *KCNQ2* variant screening. In brief, the family history was remarkable for their first child, who presented with repetitive episodes of desaturation and clonic jerks in the first 48 h of life that resolved after a loading dose of PB. She was in the NICU for 8 days without further clinical and electrographic seizures, and had a normal MRI. At 1 year of age, she had a seizure and subsequently developed neurological disability with autism features. At 5 year of age a novel heterozygous variant c.152del p.(Gly51Alafs*82) in the *KCNQ2* gene was identified. At that time mother was 19 weeks pregnant and a prenatal screening for the familial variant performed simultaneously in the fetus and both parents, revealed the *KCNQ2* variant in the fetus and in the asymptomatic father without a history of seizures in the neonatal period.

All variants were classified as pathogenic [21]. Furthermore, patient 1, 2 and 3 were previously published [11 17].

*Follow-up*

The median length of hospital stay was 15,4 days (range 6 - 45 days). Patient 1 had 2 seizure recurrences at 1 and 6 years after a febrile illness. He is seizure free till now on VPA and levetiracetam. At age 9 he has intellectual disability, no language and autistic features.

Patient 2 had seizure recurrence at 3 years after CBZ was discontinued. CBZ was reintroduced and she remains seizure free. When she was last seen at age 7, she was in 2$^{nd}$ grade of elementary school with excellent results.

Patient 3 was weaned off CBZ at age 3 and is still seizure-free at 4 years of age with a normal development.

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Patient 4 had recurrence of seizures at 15 days of life during acute SARS-CoV2 infection. When seen at 1 year of age she had global motor delay functioning at a level of 3 months. She remained seizure free on CBZ.

Patient 5 had a normal development, on CBZ, but follow-up is still limited to 8 months.

The median of follow-up was over 52 months (range 8 months – 9 years).

**Discussion**

We report 5 patients with KCNQ2/3-related epilepsy who were admitted over an 8-year period, during which the recognition of the typical aEEG pattern has led to an early and effective treatment with CBZ in four patients. Although acute provoked seizures due to hypoxic-ischemic encephalopathy, intracranial hemorrhage, arterial ischemic stroke or intracranial infection are most commonly encountered in the NICU, in a minority of cases, neonatal seizures may be the first manifestation of genetic epilepsies [2]. Early distinction from acute provoked seizures has an impact not only on treatment but also on investigations and prognostic implications. Our first patient - index case clearly shows the difficulties to diagnose in a timely manner and the consequences of ineffective treatment. A stepwise approach was followed and infection, metabolic and structural etiologies were ruled out within a short period. His distinctive ictal aEEG findings were not recognized and CBZ was not used. In fact, CBZ was not part of our standard seizure treatment protocol at that time. This resulted in a high-seizure burden and this may have contributed to a poor neurodevelopmental outcome. Genetic epilepsy was considered at 3 months of age and the results were only available at 8 months of age.

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KCNQ2/3-related epilepsy has an onset in the first days of life and presents with tonic or sequential seizures [1 2 4 6]. Recognition of the seizure semiology is very important as a guide to the workup [6 22]. In spite of a marked tonic component in all patients, the semiology did not lead to the diagnosis of genetic epilepsy at admission to the NICU. Indeed, recognizing neonatal seizures solely based on clinical semiology may be difficult [23]. All seizure types require confirmation with EEG or an aEEG [2]. The use of routine two-channel aEEG/cEEG at the bedside in the NICU has become a clinical standard monitor worldwide. It is easy to perform and does not depend on 24/7 neurophysiology support [9]. Nevertheless, several limitations are well recognised. Time compression and limited number of channels may result in suboptimal seizure detection, and artifacts can mimic seizure-like patterns in the aEEG leading to a false positive diagnosis [23 24]. Our local protocol to manage neonatal seizures recommend two-channel aEEG/cEEG or multichannel cEEG with dual channel aEEG (aEEG/cEEG) monitoring to confirm clinical seizures and monitor efficacy of treatmentContinuous monitoring was started shortly after admission in all infants and response to ASM was assessed under aEEG/cEEG monitoring. Our index case was indeed a source of learning: the ictal aEEG pattern was different from provoked seizures, there was no structural brain damage or malformations on MRI nor metabolic changes, and there was no response to the classic ASM. The recognition of a sequential clinical seizure pattern with a prominent tonic onset on aEEG/cEEG was at that time a clue to genetic epilepsy due to channelopathies [4 12]. Since then, genetic testing has been offered to all newborns in case of unknown etiology of seizures. With this methodology it is unlikely that we missed cases of KCNQ2/3 channelopathy in this time frame, that did not present the distinctive ictal aEEG pattern [11 17]. So, we considered that selection

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bias is low. The reporting literature highlighting the response to SCB [1 6-8] led us to introduce CBZ in our seizure protocol, and initiation of targeted therapy solely based on the recognition of this electroclinical phenotype while genetic testing is pending. After our index case, we were able to recognize the distinctive ictal aEEG pattern in the subsequent 4 patients and targeted therapy with CBZ was brought forward, ranging from 12h to 120 h after the 1st electroclinical seizure. This wide range could be explained in part because of the low seizure frequency in our case series and because usually neonatologists are trained to recognize a single seizure like a clear simultaneous rise in lower and upper margin rather than a predominant prolonged post-ictal suppression which was present in all [9-12]. This background post-ictal suppression is not present in acute provoked seizures nor in other genetic epilepsies such as PRRT2, KCNT1 or BRAT1 [6 17]. ASM may affect and depress the electrocortical background activity and consequently this could influence the duration of post-ictal suppression. Their effect as a potential confounder was analyzed. The possibility of having long-term aEEG trend monitoring and details on whether ASM had been administered, enhances identification of such effects on the electrocortical activity, and this was not observed. To improve the accuracy of aEEG detection in real time, when this type of ictal pattern, is suspected we recommend to confirm the ictal pattern with less time compression, such as 15cm/h and with simultaneous analysis of the raw EEG and measurement of the post-ictal suppression. The observation of a diffuse EEG flattening or attenuation following a post ictal phase has been reported in KCNQ2 [1 4 6 10-17], but this is the first time that a possible correlation between the duration of the post-ictal suppression and neurodevelopmental outcome is reported. We believe that the length of post-ictal suppression could be used as a clinical

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biomarker in future research. Besides the aEEG utility in seizure detection and monitoring treatment, evaluation of the background pattern and presence or absence of SWC could also provide additional information concerning neurodevelopmental outcome.

PB is recommended as 1st line ASM irrespective of seizure etiology, except for a family history that suggests channelopathy, then a SCB should be the 1st line ASM [25]. All seizures were timely detected in our case series and PB was the 1st line ASM in four patients, being ineffective. Patient 1 presented with pharmacoresistant seizures that evolved to a SE, and was stopped by lidocaine, that showed good efficacy for the time of the continuous infusion. Sodium channel blockers including carbamazepine, oxcarbazepine, phenytoin and lidocaine can provide seizure freedom or a significant reduction in KCNQ2/3-related epilepsy [6-8]. In a recent systematic review, PB or CBZ are the most effective ASM in SLNE and considering side effects, CBZ is suggested as 1st choice when suspicion is high. In DEE, PB was ineffective as 1st line ASM. SCB, VPA and levetiracetam were partially effective without significant differences [26]. Patient 1, CBZ was not trialled but the last ASM to introduce was VPA. In patient 2 and 4 levetiracetam was partially effective. Since our index case, the recognition progressively improved and the evolving knowledge on neonatal-onset epilepsies has changed our clinical management allowing early diagnosis and earlier use of CBZ in the NICU even prior to obtaining the genetic results with seizure control by the end of the first week of life. An overall decrease in polytherapy was noticed after our index case and CBZ was even the 1st ASM in one patient and the 2nd ASM in another one. Once again, we emphasize the recognition of the electroclinical phenotype and not to rely only on the family history to consider a channelopathy and to begin SCB as soon as

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possible. Indeed, in our case series only patient 5 had a positive family history, allowing for targeted familial frameshift variant screening in the *KCNQ2* gene and an earlier diagnosis. On the other hand, the remaining patients presented *de novo* missense *KCNQ2* pathogenic variants, and the genetic study was directed based on their electroclinical presentation.

Although the underlying causes of the differential developmental trajectories in *KCNQ2*-related disorders are not fully understood, some genotype-phenotype correlations have emerged, spanning from SLNE with transient seizures in the first months of life to DEE, characterized by neonatal onset seizures with subsequent long term developmental impairments and later-onset disability with and without seizures [4 26-28]. In our case series we may consider that patients 2,3 and 5 present a SLNE phenotype and patient 1 and 4 a DEE phenotype.

Patient 1, had a higher seizure-burden with multiple ASM and was not trialled to CBZ. The impact of seizure-burden on cognition and behavioural outcome is increasingly being recognized [29 30], and there is also concern about the side effects of ASM [31]. This could partly explain his poor neurodevelopmental outcome. However, patient 4 started CBZ at 7 days of age with rapid seizure control but at one year she also showed global motor delay. It seems that early seizure control is not enough to preclude a normal neurodevelopment. This genetic disorder may directly lead to both severe epilepsy and profound intellectual disability as two independent dimensions of the phenotype [28].

Characterizing the specific effects of each *KCNQ2* variant on channel function could help identify distinct pathogenic mechanisms, supporting genotype-phenotype correlations and potentially influencing the clinical management of these patients.

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Previous functional studies have shown that DEE-causing substitutions often induce strong loss-of-function, dominant-negative, or gain-of-function effects, in contrast to SLNE-causing variants associated with haploinsufficiency [27 32]. Additionally, taking into account patient 5 and his family history(asymptomatic father; sister with neurological disability and autism features; and patient 5 with seizures in the neonatal period but normal outcome so far), suggests the hypothesis that other factors, such as the presence of modifier genes that encode channels with direct interactions or that are part of the same pathway, may modulate the penetrance or clinical expression associated with *KCNQ2*-related disorders [33].

Therefore, gaining a comprehensive understanding of the pathogenic molecular mechanisms underlying *KCNQ2* variants and their correlation with clinical phenotypes holds significant implications for the clinical management of patients. It not only aids in accurate diagnosis and prognosis but also paves the way for targeted therapeutic strategies.

While these advances are ongoing, we raise the question whether aEEG/cEEG findings, can be of additional value to predict the neurological and developmental outcome. Patient 1 and 4 had the longest post-ictal amplitude suppression on aEEG/cEEG, no SWC and patient 4 also had a DNV background pattern. The other infants presented with a CNV pattern with SWC and shorter post-ictal amplitude suppression.

In conclusion, KCNQ2/3-related epilepsy commonly presents as sequential seizures with at times a distinctive ictal aEEG pattern and early recognition will influence treatment choice with SCB before genetic results become available. With this practical approach rapid distinction between genetic epilepsies and acute provoked seizures can be made.

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The next step is to have access to early biomarkers that could help to identify which patient will have a poor developmental outcome irrespective of seizure control. As more cases of KCNQ2/3-related epilepsy are being diagnosed prospectively and timely treated with SCB, it would be interesting to analyze whether aEEG background pattern, presence of SWC and duration of post-ictal amplitude suppression can be used as biomarkers of neurodevelopmental outcome.

Further functional studies are warranted to elucidate the precise mechanisms driving the observed genotype-phenotype relationships and potentially guide personalized treatment approaches for individuals with *KCNQ2/3* variants.

Conflicts of interest

The authors report no conflicts of interest

**References**


1. Cornet MC, Cilio MR. Genetics of neonatal-onset epilepsies. Handb Clin Neurol 2019;**162**:415-33 doi: 10.1016/B978-0-444-64029-1.00020-5 [published Online First: 2019/07/22].
2. Pressler RM, Cilio MR, Mizrahi EM, et al. The ILAE classification of seizures and the epilepsies: Modification for seizures in the neonate. Position paper by the ILAE Task Force on Neonatal Seizures. Epilepsia 2021;**62**(3):615-28 doi: 10.1111/epi.16815 [published Online First: 2021/02/02].
3. Wang HS, Pan Z, Shi W, et al. KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science 1998;**282**(5395):1890-3 doi: 10.1126/science.282.5395.1890 [published Online First: 1998/12/04].
4. Weckhuysen S, Ivanovic V, Hendrickx R, et al. Extending the KCNQ2 encephalopathy spectrum: clinical and neuroimaging findings in 17 patients. Neurology 2013;**81**(19):1697-703 doi: 10.1212/01.wnl.0000435296.72400.a1 [published Online First: 20131009].



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5. Grinton BE, Heron SE, Pelekanos JT, et al. Familial neonatal seizures in 36 families: Clinical and genetic features correlate with outcome. Epilepsia 2015;**56**(7):1071-80 doi: 10.1111/epi.13020 [published Online First: 20150515].
6. Cornet MC, Morabito V, Lederer D, et al. Neonatal presentation of genetic epilepsies: Early differentiation from acute provoked seizures. Epilepsia 2021;**62**(8):1907-20 doi: 10.1111/epi.16957 [published Online First: 2021/06/22].
7. Pisano T, Numis AL, Heavin SB, et al. Early and effective treatment of KCNQ2 encephalopathy. Epilepsia 2015;**56**(5):685-91 doi: 10.1111/epi.12984 [published Online First: 20150416].
8. Reif PS, Tsai MH, Helbig I, Rosenow F, Klein KM. Precision medicine in genetic epilepsies: break of dawn? Expert Rev Neurother 2017;**17**(4):381-92 doi: 10.1080/14737175.2017.1253476 [published Online First: 20161110].
9. Hellstrom-Westas L. Amplitude-integrated electroencephalography for seizure detection in newborn infants. Semin Fetal Neonatal Med 2018;**23**(3):175-82 doi: 10.1016/j.siny.2018.02.003 [published Online First: 20180213].
10. Hellström-Westas L, De Vries LS, Rosén I. *An Atlas of Amplitude-integrated EEGs in the Newborn (2nd ed.)*: Parthenon Publishing Group, 2009.
11. Vilan A, Mendes Ribeiro J, Striano P, et al. A Distinctive Ictal Amplitude-Integrated Electroencephalography Pattern in Newborns with Neonatal Epilepsy Associated with KCNQ2 Mutations. Neonatology 2017;**112**(4):387-93 doi: 10.1159/000478651 [published Online First: 20170920].
12. Numis AL, Angriman M, Sullivan JE, et al. KCNQ2 encephalopathy: delineation of the electroclinical phenotype and treatment response. Neurology 2014;**82**(4):368-70 doi: 10.1212/WNL.0000000000000060 [published Online First: 20131226].
13. Dilena R, Mauri E, Di Fonzo A, et al. Case Report: Effect of Targeted Therapy With Carbamazepine in KCNQ2 Neonatal Epilepsy. Front Neurol 2022;**13**:942582 doi: 10.3389/fneur.2022.942582 [published Online First: 20220714].
14. Kazazian V, Selvanathan T, Chau V, Tam EWY, Miller SP. Pearls & Oy-sters: Genetic Epilepsy: An Important Cause of Neonatal Seizures in Infants With Complex Congenital Heart Disease. Neurology 2022 doi: 10.1212/WNL.0000000000201229 [published Online First: 20220830].
15. Lee IC, Chang MY, Liang JS, Chang TM. Ictal and interictal electroencephalographic findings can contribute to early diagnosis and prompt treatment in KCNQ2-associated epileptic encephalopathy. J Formos Med Assoc 2021;**120**(1 Pt 3):744-54 doi: 10.1016/j.jfma.2020.08.014 [published Online First: 20200827].
16. Lee IC, Hong SY, Weng YH, Chen YT. Amplitude Integrated Electroencephalography and Continuous Electroencephalography Monitoring Is Crucial in High-Risk Infants and Their Findings Correlate With Neurodevelopmental Outcomes. Front Pediatr 2021;**9**:691764 doi: 10.3389/fped.2021.691764 [published Online First: 20210803].
17. Pijpers JA, Au PYB, Weeke LC, et al. Early recognition of characteristic conventional and amplitude-integrated EEG patterns of seizures in SCN2A and KCNQ3-related epilepsy in neonates. Seizure 2023;**110**:212-19 doi: 10.1016/j.seizure.2023.06.016 [published Online First: 20230622].
18. Shellhaas RA, Chang T, Tsuchida T, et al. The American Clinical Neurophysiology Society's Guideline on Continuous Electroencephalography Monitoring in Neonates. J Clin Neurophysiol 2011;**28**(6):611-7 doi: 10.1097/WNP.0b013e31823e96d7.
19. Scher MS, Hamid MY, Steppe DA, Beggarly ME, Painter MJ. Ictal and interictal electrographic seizure durations in preterm and term neonates. Epilepsia 1993;**34**(2):284-8 doi: 10.1111/j.1528-1157.1993.tb02412.x.
20. Lhatoo SD, Faulkner HJ, Dembny K, Trippick K, Johnson C, Bird JM. An electroclinical case-control study of sudden unexpected death in epilepsy. Ann Neurol 2010;**68**(6):787-96 doi: 10.1002/ana.22101.

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21. 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**(5):405-24 doi: 10.1038/gim.2015.30 [published Online First: 20150305].
22. Nunes ML, Yozawitz EG, Zuberi S, et al. Neonatal seizures: Is there a relationship between ictal electroclinical features and etiology? A critical appraisal based on a systematic literature review. Epilepsia Open 2019;**4**(1):10-29 doi: 10.1002/epi4.12298 [published Online First: 20190125].
23. Malone A, Ryan CA, Fitzgerald A, Burgoyne L, Connolly S, Boylan GB. Interobserver agreement in neonatal seizure identification. Epilepsia 2009;**50**(9):2097-101 doi: 10.1111/j.1528-1167.2009.02132.x [published Online First: 20090601].
24. Rakshasbhuvankar A, Paul S, Nagarajan L, Ghosh S, Rao S. Amplitude-integrated EEG for detection of neonatal seizures: a systematic review. Seizure 2015;**33**:90-8 doi: 10.1016/j.seizure.2015.09.014 [published Online First: 20150926].
25. Pressler RM, Abend NS, Auvin S, et al. Treatment of seizures in the neonate: Guidelines and consensus-based recommendations-Special report from the ILAE Task Force on Neonatal Seizures. Epilepsia 2023 doi: 10.1111/epi.17745 [published Online First: 20230901].
26. Falsaperla R, Criscione R, Cimino C, Pisani F, Ruggieri M. KCNQ2-Related Epilepsy: Genotype-Phenotype Relationship with Tailored Antiseizure Medication (ASM)-A Systematic Review. Neuropediatrics 2023 doi: 10.1055/a-2060-4576 [published Online First: 20230515].
27. Miceli F, Millevert C, Soldovieri MV, et al. KCNQ2 R144 variants cause neurodevelopmental disability with language impairment and autistic features without neonatal seizures through a gain-of-function mechanism. EBioMedicine 2022;**81**:104130 doi: 10.1016/j.ebiom.2022.104130 [published Online First: 20220630].
28. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017;**58**(4):512-21 doi: 10.1111/epi.13709 [published Online First: 20170308].
29. Kharoshankaya L, Stevenson NJ, Livingstone V, et al. Seizure burden and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy. Dev Med Child Neurol 2016;**58**(12):1242-48 doi: 10.1111/dmcn.13215 [published Online First: 20160906].
30. Payne ET, Zhao XY, Frndova H, et al. Seizure burden is independently associated with short term outcome in critically ill children. Brain 2014;**137**(Pt 5):1429-38 doi: 10.1093/brain/awu042 [published Online First: 20140304].
31. Pressler RM, Lagae L. Why we urgently need improved seizure and epilepsy therapies for children and neonates. Neuropharmacology 2020;**170**:107854 doi: 10.1016/j.neuropharm.2019.107854 [published Online First: 2019/11/22].
32. Vanoye CG, Desai RR, Ji Z, et al. High-throughput evaluation of epilepsy-associated KCNQ2 variants reveals functional and pharmacological heterogeneity. JCI Insight 2022;**7**(5) doi: 10.1172/jci.insight.156314 [published Online First: 20220308].
33. Bartolini E. Inherited Developmental and Epileptic Encephalopathies. Neurol Int 2021;**13**(4):555-68 doi: 10.3390/neurolint13040055 [published Online First: 20211103].

Table 1

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| | Patient 1 (index case)/2014 | Patient 2/2015 | Patient 3/2019 | Patient 4/2022 | Patient 5/2022 |
|---|---|---|---|---|---|
| Family history | No | No | No | No | Yes/older sister |
| GA/sex/ Apgar scores | Term/ male/9/10 | Term/ female/9/10 | Term/ female/9/10 | Term/ female/9/10 | Term/ female/9/10 |
| Length of hospital stay | 45 days | 10 days | 6 days | 10 days | 6 days |
| Seizures | | | | | |
| Age at seizure onset | 36h | 36h | 48h | 20h | 30h |
| Semiology | tonic asymmetric with head deviation, eye blinking, facial flushing followed by unilateral clonic jerk; sometimes bilateral tonic posturing of upper and lower extremities, salivation and desaturation | tonic asymmetric, head deviation, unilateral clonic jerk and desaturation | bilateral tonic posturing of upper and lower limbs, retrocollis, facial flushing, salivation followed by unilateral clonic jerk | bradycardia, bilateral tonic posturing of upper and lower extremities, desaturation followed by high pitched cry, On re-admission: tonic asymmetric, head deviation, eye blinking, high pitched cry | Tonic asymmetric, facial flushing, salivation, desaturation |
| Seizure frequency evolution | Repetitive, status epilepticus, single | Repetitive, single | Single | Single, repetitive | Single |
| Neurophysiologic evaluation | | | | | |
| Recognition of typical ictal aEEG | No | yes | yes | yes | yes |
| Interictal aEEG | CNV | CNV | CNV | DNV | CNV |
| Sleep wake cycle present | no | yes | yes | no | yes |

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| | | | | | |
|---|---|---|---|---|---|
| Unilateral Ictal pattern present | yes | yes | yes | no | yes |
| Duration (sec) of post-ictal suppression of first recorded seizure | 174 s | 110 s | 96 s | 192 s | 52s |
| Duration (sec) of post-ictal suppression prior to CBZ (mean±sd) | n/a | 117 (72-112)<br>89±17 | 102.2 (81-118)<br>100±13 | 141.3 (77-192)<br>141±35 | 55.5 (50-63)<br>55±5 |
| Treatment | | | | | |
| ASM (order of administration) | PB, pyridoxine, LEV, MDZ, lidocaine, CZP, TPM, VPA | PB, LEV, pyridoxine, CBZ | PB, CBZ | PB, Lev, CBZ | CBZ |
| ASM response | Lidocaine effective | Lev partially effective; CBZ effective | CBZ effective | Lev partially effective; CBZ effective | CBZ effective |
| Time of CBZ administration after recognition of ictal aEEG | n/a | 96h | 18h | 120h | 12h |
| Age at SZ control | 30 d | 5.5 d | 6d | 7d | 2d |
| CBZ dosage | n/a | 15mg/kg/day; 8/8h | 20mg/kg/day; 8/8h | 20mg/kg/day; 8/8h | 20mg/kg/day; 8/8h |
| Other ancillary tests | | | | | |
| Brain MRI | Normal | Normal | Normal | Normal | n/a |
| Genetic test | *KCNQ2* gene sequencing | *KCNQ2* gene sequencing | Epilepsy NGS panel | Epilepsy NGS panel | Testing for *KCNQ2* familial variant |
| Genotype | *de novo KCNQ2:* c.830C>T, p.(Thr277Ile) | *de novo KCNQ2:* c.1657C>T, p.(Arg553Trp) | *de novo KCNQ3:* c.956A>G, p.(Tyr319Cys) | *de novo KCNQ2:* c.1742G>A, p.(Arg581Gln)) | inherited *KCNQ2:* c.152del, p.(Gly51Alafs*82) |
| Outcome | | | | | |
| Neurological examination | Lack of visual | Lack of visual fixation | Normal | Lack of visual fixation | Normal |

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| | | | | | |
|---|---|---|---|---|---|
| at discharge | fixation Hypotonia | Axial hypotonia | | Axial hypotonia | |
| Sz outcome | Recurrence at 1 and 6 years during febrile illness | Recurrence at 3 years upon discontinuation of CBZ | Sz-free No recurrence | Recurrence at 29 days, during SARS-Cov2 infection | Sz-free No recurrence |
| Current ASM at last FU | VPA and LEV | CBZ | none | CBZ | CBZ |
| Developmental outcome/age at last FU | Profound ID. Autism. 9 years | Normal/7 years | Normal /4 years | Global psychomotor delay. Developmental plateu at age 3 months/1 year | Normal/8 months |

CBZ, carbamazepine; CNV, continuous normal voltage; CZP, clonazepam; DNV, discontinuous normal voltage; follow up, FU; ID, intelectual disability; LEV, levetiracetam; MDZ, midazolam; n/a, not applicable; NGS, new generation sequencing; PB, phenobarbital; SZ, seizures; TPM, topiramate; VPA, valproic acid.

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