

Case Report

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Precision medicine in early-onset epilepsy: the KCNQ2 paradigm

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Abstract

The identification of the genetic causes and the underlying pathogenic mechanisms in early-onset epilepsies has proved to be essential in improving the efficacy of therapeutic decisions and the overall patient management, especially in the era of precision medicine. We report an infant presenting with a cluster of focal motor seizures with autonomic manifestations at day 3 of life. Electroencephalograms showed multifocal epileptic abnormalities and a burst-suppression pattern. Neurological examination showed poor visual fixation and hypotonia. Neuroimaging was normal. Seizures remitted with phenytoin and were well-controlled after the switch to oral carbamazepine. In the hypothesis of a genetic etiology, next-generation sequencing panel for epileptic encephalopathies was performed and identified a *de novo* missense mutation in *KCNQ2*: c.1742G>A; p.(Arg581Gln) (NM_172107.2). This case report highlights the importance of the early recognition of the electroclinical phenotype and the detection of the underlying genetic cause in the implementation of "tailored" therapies in early-onset genetic epilepsies.

Keywords: *KCNQ2*, early-onset epilepsy, developmental and epileptic encephalopathy, next-generation sequencing, precision medicine, sodium channel blockers



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INTRODUCTION

Epilepsy is one of the most common neurological disorders. It is more frequent in infancy with an incidence of approximately 70 per 100,000 children younger than 2 years^[1]. Approximately 70%-80% of childhood epilepsy cases are caused by one or more genetic alterations, which comprise both monogenic and polygenic factors^[2]. While the majority of seizures occurring during the neonatal period are caused by acute brain injury^[3], around 13% of neonates suffer from neonatal-onset epilepsy^[4] with a specific genetic cause, usually showing up in the first days of life.

Neonatal-onset genetic epilepsies can be classified into three large groups: genetic conditions associated with structural brain anomaly, genetic conditions associated with metabolic disorders, and genetic conditions resulting in functional cortical anomalies^[3]. The latter group includes disorders of cell signalling (e.g., *CDKL5*, *BRAT1*), disorders of synaptic transmission (e.g., *STXBP1*), and channelopathies (e.g., *KCNQ2*, *KCNT1*, *SCN2A*)^[3]. Pathogenic variants in *KCNQ2* are the most common genetic cause of neonatal seizures among the channelopathies^[5]. Dominant mutations in this gene lead to different epileptic disorders, including benign epilepsy and developmental and epileptic encephalopathy (*KCNQ2*-related DEE)^[6]. The clinical features suggestive of *KCNQ2*-related DEE include seizure onset in the first week of life with severely abnormal electroencephalography background activity, absence of structural lesions in the brain, and mild to severe developmental delay^[6,7].

Our understanding of the etiology and pathophysiology of DEE has considerably improved thanks to Next Generation Sequencing (NGS). This technique has become faster and more accessible in recent years^[8], allowing the development of treatments addressing the precise molecular causes of the disease^[8]. Early genetic diagnosis is critical to avoid further potentially invasive diagnostic tests and to make appropriate therapeutic decisions. The case of *KCNQ2* related-epilepsy is paradigmatic, as supported by the importance of the early recognition of the specific electroclinical phenotype and the use of sodium channel blockers (SCB) in this disorder^[9].

We describe a boy with *KCNQ2*-related DEE presenting with neonatal-onset seizures; the epilepsy gene-panel revealed a *de novo* *KCNQ2* variant: c.1742G>A; p.(Arg581Gln) (NM_172107.2). We highlight the immediate and remarkable response to the treatment with SCB in this patient, further supporting the role of this therapeutic approach in *KCNQ2* disorders.

CASE REPORT

This 7-month-old infant was the first child from unrelated, healthy parents. Family history was negative for epilepsies, mental retardation, and hereditary diseases, but it was positive for febrile seizures on the maternal side. He was born at term by urgent caesarean section for deceleration of the heartbeat, after an uneventful pregnancy. Fetal karyotype was normal. APGAR score was 8 at 1 min and 9 at 5 min. Auxological parameters at birth were: weight 2,650 g (-2.2 Standard Deviation - SD, SGA), length 52 cm (+1.13 SD), and occipital frontal circumference (OFC) 36 cm (+1.41 SD). Transient neonatal hypoglycemia was found, but the perinatal course was otherwise uneventful.

At day 3, the baby presented recurrent seizures, lasting around one minute and characterized by tonic posturing with flexion of the lower limbs, ocular retroversion, horizontal nystagmus, perioral cyanosis, automatic sucking movements, and apnea (with SaO₂ < 45). He was hospitalized in the Neonatal Intensive Care Unit and a serial electroencephalographic (EEG) monitoring was performed. The first EEG showed rare paroxysmal abnormalities in the left hemisphere. Due to the daily recurrence of clustered focal motor and autonomic seizures, the patient was treated with intravenous phenobarbital (PB) at the initial dose of 20 mg/kg up to the maintenance dose of 5 mg/kg twice daily, with no effect. At day 6, levetiracetam

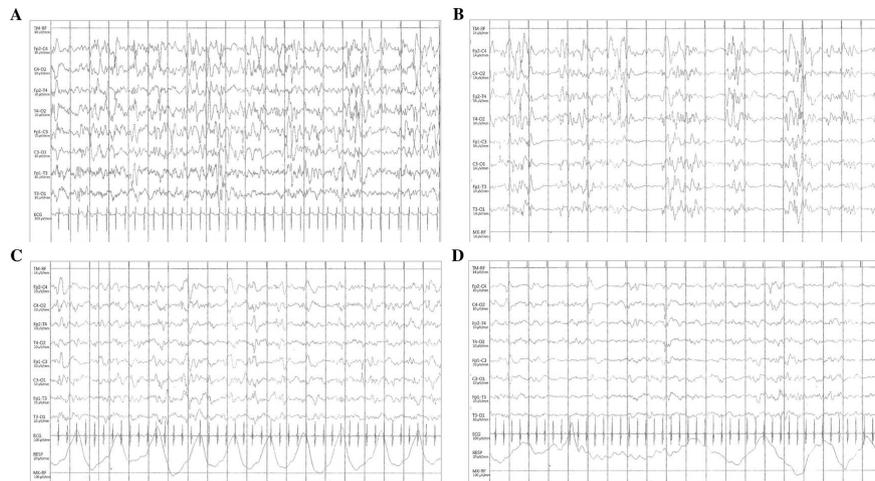


Figure 1. EEG findings in the patient. A: EEG at 13 days showing multifocal epileptiform abnormalities; B: EEG at 18 days shows suppression burst activity; C: EEG during sleep at 25 days after the introduction of sodium channel blockers reveals an attenuation of multifocal epileptiform abnormalities; D: EEG in wake with sporadic epileptiform abnormalities and monomorphic theta-delta rhythms, combined with slow bifrontal graphoelements. All EEG shown are in a reduced montage with setting the low-frequency time constant at 0.1 seconds, high-frequency filter at 50 Hz (except in frame B where it is 15 Hz), notch filter at 50 Hz, sensitivity at 10 μ V/mm (except in frame B: 14 μ V/mm), paper speed is 20 sec per epoch

(LEV) (16 mg/kg/twice per day) was added-on with partial response. Neurological examination revealed poor visual fixation, generalized hypotonia, right head deviation, and opisthotonus. Biochemical workup (serum electrolytes and ammonium, glucose level, coagulation profile, plasma, and urinary amino acids, acylcarnitine, and urinary organic acids) and extensive screening for possible infections in blood and cerebrospinal fluid were unremarkable. Electrocardiogram, echocardiogram, abdomen ultrasonography, and auditory brainstem evocative potentials were normal. Brain magnetic resonance imaging, including spectroscopy and angio sequences, was unremarkable at age 13 days.

Seizures recurred with axial hypertonia, extension of the four limbs, deviation of the head to the right side, perioral cyanosis, and occasional desaturation. A second EEG at 13 days of age showed relevant multifocal epileptiform abnormalities and no significant change was observed after a trial with 100 mg pyridoxine bolus [Figure 1A]. Furthermore, seizures persisted despite the administration of intravenous midazolam at maximum dosages and pyridoxine, in add-on with PB and LEV. Likewise, the introduction of clobazam (5 mg/die) was ineffective. At 18 days, the EEG recording confirmed a significant multifocal paroxysmal activity. A burst suppression activity was also observed [Figure 1B]; moreover, amplitude-integrated EEG (aEEG) monitoring showed a pattern with “peaks” and subsequent depression.

Thus, genetically determined epilepsy with neonatal-onset was suspected. After written informed consents obtained from the parents, DNA was extracted from the peripheral blood of the trio and NGS-based panel including 54 genes involved in epileptic encephalopathies was performed at twenty days of life (full list of genes is available on [Supplementary Table 1](#)).

Intravenous phenytoin (PHT) was started and then switched to oral, gradually adjusting the dosages based on the blood levels. The final dosage was 40 mg/kg/day divided into three administrations. This therapy did control the seizures and the other antiepileptic medications (VB₆, LEV, PB and benzodiazepines) were gradually withdrawn. The EEG recording at 25 days of age still showed multifocal epileptiform activity in sleep, with alternating trends [Figure 1C]. These abnormalities were attenuated during wake and were associated with medium voltage monomorphic theta-delta rhythms with slow bifrontal graphoelements. At age 1 month, the EEG revealed better-organized background activity and a reduction in the epileptic

activity [Figure 1D]. At 36 days of age, PHT blood levels were found to be inappropriately low (0.9 µg/mL, reference range: 10-20 µg/mL) and electroclinical worsening was observed. Accordingly, the PHT dosage was increased up to 45 mg/kg/day, in three daily administrations. Background EEG activity was replaced by abnormal activity which included spikes and polyspikes, often followed by slow waves of medium amplitude, which were synchronous and asynchronous in both hemispheres and more prominent in the frontal derivations. Sometimes these abnormalities were more diffuse. Additional EEGs performed at 2 months of life showed a significant improvement. There were rare epileptic abnormalities with multifocal expression and predominant posterior origin.

Due to the confirmation of a heterozygous missense mutation in *KCNQ2* [c.1742G>A; p.(Arg581Gln)] absent in the parents (*de novo* origin), we introduced carbamazepine (CBZ) at the initial dosage of 9 mg/Kg/day, progressively increasing up to an adequate dosage for body weight. This change in the therapeutic regimen resulted in good seizures control. Two further EEGs (at the age of 70 and 85 days), showed a lack of organization and multifocal epileptic abnormalities. The baby was discharged from hospital at the age of 3 months. He was seizure-free with CBZ monotherapy.

At 7 months of age, the patient experienced daily episodes of apnea and cyanosis preceded by crying. Video-EEG showed multifocal epileptic abnormalities and confirmed the epileptic nature of these episodes. Furthermore, the CBZ blood levels were 2.4 µg/mL (reference range: 8-12 µg/mL). The dosage was thus increased (16.7 mg/kg/die) with subsequent good seizures control. Physical examination revealed frontal bossing, irritability, mild hypertonia, vocalizations, and inconstant visual fixation. Psychomotor delay was observed, as he was not able to sit unassisted. Auxological parameters were: weight of 7.2 kg (-1.64 SD), length of 64 cm (-2.05 SD), OFC 46 cm (+1.75 SD). Head ultrasound showed a 6 mm dilation of the periencephalic spaces of the cranial vault.

DISCUSSION

KCNQ2 is located on chromosome 20q13.33 and encodes the potassium channel subunit $K_{v}7.2$, that forms heterotetrameric voltage-gated potassium channels together with $K_{v}7.3$ subunit (encoded by *KCNQ3*). These channels are widely expressed in the brain and transport potassium ions along their concentration gradient, underlying the M-current. This slowly activating, non-inactivating potassium conductance inhibits neuronal excitability^[10]. Pathogenic variants in *KCNQ2* leading to loss of function cause two different epilepsy phenotypes^[6] Truncating and missense variants, likely resulting in haploinsufficiency, are associated with benign familial neonatal epilepsy (BFNE) whereas missense variants and small in-frame deletions, resulting in dominant-negative effect, are associated with DEE^[6,11]. Hereditary or *de novo* BFNE-associated *KCNQ2* variants are distributed along with the channel protein and are more common in the intracellular domain between S2 and S3^[12]. The mutations in *KCNQ2* in encephalopathic patients are instead found in four functionally relevant protein domains: the domain of the voltage sensor, the pore, the proximal regions C-terminus, and calmodulin-binding helix region B^[7,12]. The variant detected in our case is reported in the ClinVar database in an individual with neonatal/infantile-onset epileptic encephalopathy (#ClinVar, variation ID 21769, <https://www.ncbi.nlm.nih.gov/clinvar/variation/21769/>). This variant involves an evolutionarily conserved amino acid located in the C-terminus domain, likely resulting in an impaired protein function.

The recognition of seizures in a new-born can be challenging as clinical manifestations may be subtle. The EEG background plays a relevant role in the identification of patients with benign epilepsy or severe epilepsy. Indeed, video-EEG is a powerful tool for the early recognition of distinct electro-clinical aetiology-specific phenotypes. This is further supported by what has been observed in *KCNQ2*-related epilepsies, *KCNT1* encephalopathy, and *STXBP1* encephalopathy^[13]. In our patient, the neonatal EEG background showed multifocal epileptic abnormalities with random attenuation alternating with burst suppression pattern. Moreover, aEEG showed a pattern that has been recently suggested as a signature in neonates with *KCNQ2*-related disorders^[14].

Deep phenotypic characterization played a relevant role in the choice of the most appropriate genetic test in our case. Genetic testing should be performed as early as possible, especially in patients showing some specific electro-clinical phenotypes (e.g., *KCNQ2* or *KCNT1*), to recognize potentially treatable disorders^[15].

In our patient, genetic testing was performed within the first month of life and the results were obtained at age 2 months, revealing the *de novo* missense mutation in *KCNQ2* and allowing a “targeted” treatment.

The role of a precision medicine approach in neonatal seizures with known etiology is becoming more and more relevant in recent years^[13,16]. In consideration of the good electro-clinical response to SCB, our suspicion was focused on *SCN2A*, *SCN8A*, and *KCNQ2*.

Voltage-gated sodium channels and KCNQ potassium channels co-localize at the neuronal membrane and a modulating effect of SCB on both channels has been suggested^[17]. The use of broad-spectrum anti-epileptic drugs in *KCNQ2*-related epilepsy has been suggested^[9]. The most common drug used in “benign” phenotypes was PB, followed by SCB^[9]. In general, patients with a mutation in *KCNQ2* suffering from B(F)NE become seizure-free spontaneously in the first month of life or between the 6 months and 1 year of age. Accordingly, the International League Against Epilepsy recommends the use of the term “self-limiting” instead of “benign”^[18]. Therefore, the large use of PB is most likely explained by the common use of this drug as standard therapy in neonatal seizures^[9]. CBZ and PHT are instead considered appropriate as first-line treatment in *KCNQ2*-related encephalopathy, regardless of the underlying pathogenic variant^[9]. Also, lacosamide improved seizures frequency in the early-onset epileptic encephalopathy^[9]. However, functional studies and additional clinical observations are required before it can be considered a possible target therapy^[19].

The finding that retigabine opens the K_{v7} potassium channel sparked the interest in its use in patients with a *KCNQ2* mutation and it was considered the first specific anti-seizure drug for KCNQ-related epilepsy. Despite preliminary promising results, especially when the drug was administered during early development (such as improvement of seizure frequency and development in patients treated before the age of 6 months)^[12], its prescription was questioned because long-term treatment was characterized by several side effects (e.g., blue discolouration of skin and retina)^[20].

Another possible drug in patients with abnormal KCNQ function is pyridoxine. Indeed, some patients with *KCNQ2*-encephalopathy respond to this vitamin but the pathophysiology of mechanisms behind this response to pyridoxine is not completely understood^[21].

In our patient, we switched to CBZ after the dramatic effect of PHT treatment on seizure control because of the safer drug profile of CBZ within the SCB group. Indeed, other SCBs present several disadvantages, as shown by the adverse event profile of PHT (purple glove syndrome, cerebellar toxicity, and cardiac arrhythmias) or long-lasting dose increments of lamotrigine^[9].

Current treatments in *KCNQ2*-related epilepsy mainly focus on seizure control, but the early genetic diagnosis will be more and more relevant in the choice of the most appropriate therapeutic regimen, especially considering the progressive introduction of new therapies.

Advances in the knowledge of the genetic basis of early-onset epilepsies have opened the way for precision medicine approaches in this category, which had been long considered as a single entity. In *KCNQ2*-related encephalopathy, seizures may remit, but mild to profound global developmental delay may occur^[18]. Therefore, it is mandatory that in the future, clinicians will consider to run genetic testing as part of the initial workup, with higher priority for neonates, so that the results can be incorporated in the early

treatment decisions. This would, for instance, allow selecting SCBs from the beginning and avoid the usual trial-and-error approach that was also applied in our patient.

DECLARATIONS

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Authors' contributions

Drafted the manuscript: Amadori E, Brolatti N

Collected data: Brolatti N, Marchese F, Vari MS

Analyzed and interpreted the electroclinical data: Amadori E, Marchese F, Vari MS

Analyzed genetic data: Madia F

Critically reviewed the manuscript: Scala M, Ramenghi LA, Minetti C, Striano P

Availability of data and materials

The datasets generated for this study can be found in Pediatric Neurology and Muscular Diseases Unit and Neonatal Intensive Care Unit of 'G. Gaslini' Institute.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participation

Not applicable.

Consent for publication

Not applicable.

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