

DOUTORAMENTO

NEUROCIÊNCIAS

***The role of aEEG in diagnosis and management of
KCNO2/3 and SCN2A - related neonatal epilepsies***

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To my beloved daughters, Carol and Magui.
Thank you for being my refuge in every storm.
I am grateful for the wonderful moments we shared together.
Your smiles truly warm my heart.

To my sister of the heart, Cris.
Your presence in my life is a gift beyond words.

To my friends.
Thank you for being there when I most need you.

In loving memory of my parents, Isaura and Acácio, and of my
adoptive father, José Eduardo, whose love, guidance, and
strength continue to inspire me every day.

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ABBREVIATIONS

APS	acute provoked seizures
AS	active sleep
ASM	antiseizure medication
CFM	cerebral function monitor
CHD	congenital heart disease
EEG	electroencephalography
	aEEG amplitude-integrated EEG
	cEEG conventional video EEG
EIDEE	early infantile developmental and epileptic encephalopathy
HIE	hypoxic-ischemic encephalopathy
ILAE	international league against epilepsy
MRI	magnetic resonance imaging
NICUs	neonatal intensive care units
PGES	postictal generalized EEG suppression
QS	quiet sleep
RCT	randomized controlled trial
SCB	sodium channel blocker
SLNE	self limited neonatal epilepsy
SUDEP	sudden unexpected death in epilepsy
SWC	sleep-wake cycling
VUS	variants of unknown significance
WES	whole exome sequencing

ABSTRACT

In the past few years, our understanding of seizures in neonates has greatly improved with the advent of prolonged bedside video-electroencephalographic monitoring in the neonatal intensive care units. Although it is considered the gold standard for seizure diagnosis, the resource intensity of video-EEG as allowed the widespread use of amplitude-integrated EEG, which is a simplified bedside neurophysiology tool displaying two channels of EEG in a filtered and compressed manner. Also, the likelihood of reaching an aetiological diagnosis is expected to be high thanks to several advances in neuroimaging, metabolic and genetic testing. It has been recognized that, in about 15% of neonates, seizures are the first symptom of neonatal epilepsy, that may be due to a structural brain malformation, inborn errors of metabolism, or genetic defects. Determination of the seizure etiology is critical to enable provision of specific treatment and prognosis. One of the most relevant findings regarding neonatal epilepsy, such as *KCNQ2*, *KCNQ3* and *SCN2A*, which regulate the function of potassium and sodium channels, is the notable effectiveness of sodium channel blockers, like carbamazepine.

This work aimed to substantiate the clinical values of aEEG in the diagnosis and management of these genetic epilepsies.

First, in a multicenter retrospective study, of 9 newborns with *KCNQ2* variants, we analyzed aEEG features concerning the seizure pattern and we were able to recognize a distinctive ictal aEEG pattern, and provide an electroclinical phenotype that guided genetic testing and targeted treatment with oral carbamazepine. Following this initial work, we focused on *KCNQ3*- and *SCN2A*- pathogenic variants and in a multicentre descriptive study of 13 newborns, we observed the same electroclinical phenotype presented in *KCNQ2*-related epilepsy, extending this unique feature to other channelopathies. Also, the recognition of this pattern in *KCNQ3* and *SCN2A* prompted targeted treatment with sodium channel blockers, while genetic results were pending. Finally, we performed a comprehensive analysis of the aEEG concerning aEEG ictal pattern, including the postictal amplitude suppression, background pattern and sleep wake cycle in *KCNQ2/3*-related epilepsy, exploring possible biomarkers of neurodevelopment. We also quantified the time of recognition of the distinctive aEEG ictal pattern and observed seizure response to antiseizure medication under aEEG monitoring. Once again, we were able to demonstrate that aEEG can be a valuable tool at bedside in the early diagnosis and management of *KCNQ2/3*- related epilepsy.

aEEG is a complementary tool to cEEG in the diagnosis and management of genetic epilepsies. *KCNQ2/3* and *SCN2A* - related epilepsies commonly present with a distinctive ictal aEEG pattern and early recognition can guide the choice of treatment with carbamazepine, even before genetic test results are available.

KEY-WORDS

Newborn; amplitude-integrated EEG; *KCNQ2/3* and *SCN2A*-related epilepsies; carbamazepine

RESUMO

Nos últimos anos, a nossa compreensão das crises epiléticas em recém-nascidos melhorou significativamente com o advento da monitorização prolongada por vídeo-eletroencefalograma (vídeo-EEG) à cabeceira do doente nas unidades de cuidados intensivos neonatais. Embora seja considerado o padrão-ouro para o diagnóstico de crises, a exigência de recursos do vídeo-EEG permitiu a ampla utilização do electroencefalograma de amplitude integrada (aEEG), uma ferramenta simplificada de neurofisiologia à cabeceira, que exhibe dois canais de EEG de forma filtrada e comprimida. Além disso, a probabilidade de se alcançar um diagnóstico etiológico é elevada graças aos avanços nas áreas da neuroimagem, testes metabólicos e genéticos. Está reconhecido que, em cerca de 15% dos recém-nascidos, as crises são a primeira manifestação de epilepsia neonatal, que pode dever-se a uma malformação estrutural do cérebro, erros inatos do metabolismo ou defeitos genéticos. A determinação da etiologia das crises é fundamental para permitir a administração de um tratamento específico e estabelecer o prognóstico. Uma das descobertas mais relevantes no contexto da epilepsia neonatal, como nos casos associados aos genes *KCNQ2*, *KCNQ3* e *SCN2A*, que regulam a função dos canais de potássio e sódio, é a notável eficácia dos bloqueadores dos canais de sódio, como a carbamazepina, no controlo das crises epiléticas.

Este trabalho teve como objetivo demonstrar o valor clínico do aEEG no diagnóstico e tratamento destas epilepsias genéticas.

Primeiramente, num estudo retrospectivo multicêntrico com 9 recém-nascidos com variantes no gene *KCNQ2*, analisámos as características do aEEG e conseguimos reconhecer um padrão ictal distinto no aEEG, fornecendo um fenótipo electroclínico que orientou a realização de testes genéticos e o início de tratamento direcionado com carbamazepina oral. Após este trabalho inicial, focámo-nos nas variantes patogénicas dos genes *KCNQ3* e *SCN2A* e, num estudo descritivo multicêntrico envolvendo 13 recém-nascidos, observámos o mesmo fenótipo electroclínico presente na epilepsia associada ao *KCNQ2*, alargando esta característica única a outras canalopatias. Além disso, o reconhecimento deste padrão do aEEG nos genes *KCNQ3* e *SCN2A* levou ao início do tratamento direcionado com bloqueadores dos canais de sódio, enquanto os resultados genéticos ainda estavam pendentes. Finalmente, realizámos uma análise detalhada do aEEG, com a avaliação da supressão pos-ictal, da actividade de base e a existência do ciclo sono-vigília numa coorte de recém-nascidos com variantes patogénicas dos genes *KCNQ2/3*, explorando possíveis biomarcadores de neurodesenvolvimento. Quantificámos ainda o tempo necessário para o reconhecimento do padrão ictal distinto no aEEG e sob monitorização aEEG observamos a resposta aos fármacos anti- crise epilética. Mais uma vez, conseguimos demonstrar que o aEEG pode ser uma ferramenta valiosa à cabeceira, no diagnóstico precoce e tratamento da epilepsia relacionada com os genes *KCNQ2/3*.

O aEEG é uma ferramenta complementar ao cEEG no diagnóstico e tratamento das epilepsias genéticas. As epilepsias relacionadas com *KCNQ2/3* e *SCN2A* apresentam frequentemente um padrão ictal distinto no aEEG e o seu reconhecimento precoce pode orientar a escolha do tratamento com carbamazepina, mesmo antes de estarem disponíveis os resultados dos testes genéticos.

PALAVRAS-CHAVE

Recém-nascido, electroencefalograma de amplitude integrada (aEEG), epilepsia associada aos genes *KCNQ2/3* e *SCN2A*, carbamazepina.

1. INTRODUCTION

INTRODUCTION

A - Seizures in the newborn

1. Historical perspective

Seizures in the neonatal period are a distressing and potentially life-threatening condition that needs prompt recognition and treatment⁽¹⁾. They are a common neurological emergency in the newborn, with an estimative incidence of 1-3 per 1000 live births in term infants and are more common in preterm infants with high mortality and neuromorbidity^(2, 3).

Acute brain injury like hypoxic-ischemic encephalopathy (HIE), stroke, intracranial infection or acute metabolic derangements explains the vast majority of seizures in the neonatal period^(1, 4). This group is classified as acute provoked seizures (APS), as seizures are seen following a brain insult, occurring up to 72 h after birth^(4, 5). Usually, seizures stop a few days after the acute brain injury, regardless of the therapeutic intervention, with no need to further continue antiseizure medication (ASM) after discharge, regardless of magnetic resonance imaging (MRI) and electroencephalography (EEG) results^(6, 7). Recently, the advances in neuroimaging, metabolic and genetic testing have clarified our understanding of seizures in neonates and identified an increasing number of underlying causes. As opposed to APS, it has been recognized that, in about 15% of neonates, seizures are the first symptom of neonatal epilepsy⁽⁸⁾ that may be due to a structural brain malformation, inborn errors of metabolism, or genetic defects⁽⁸⁾. They usually have a poor response to ASM that cannot be withdrawn at the time of hospital discharge⁽⁵⁾.

Historically, the diagnosis of seizures in neonates was based almost exclusively on visually recognizable clinical events⁽⁹⁾. During the early 1960s, studies correlating abnormalities in the EEG of newborns with clinical seizures have been published and since then the use of EEG has become more common and contributed to the understanding of seizures in newborn in terms of physiology, diagnosis, management and prognosis^(10, 11). Indeed, the use of prolonged EEG monitoring with simultaneous observation, either by video or direct observation, has clarified very important issues concerning the identification and management of seizures in newborn namely:

- (a) some motor and behavioral phenomena previously classified as seizures do not have an electrographic correlation⁽¹²⁾,
- (b) sometimes, electrographic seizures are not accompanied by clinical symptoms, and are classified as electrographic seizures only⁽¹³⁾,
- (c) ASM can cause "uncoupling" of clinical and EEG features leading to an electroclinical dissociation⁽¹⁴⁾.

Although, conventional video-EEG (cEEG) monitoring is the gold standard for the diagnosis of seizures, not all neonatal intensive care units (NICUs) have access to cEEG especially on a 24 hours basis. Therefore, amplitude-integrated EEG (aEEG) is widely used as a standard monitoring tool in many NICUs⁽¹⁵⁾. The International League Against Epilepsy (ILAE) recommends “In situations when and where full EEG is not readily available, aEEG may be used with co-registration of raw EEG channels, although its limitations are well recognized”⁽⁴⁾. Also, the new ILAE neonatal seizure classification includes electrographic-only seizures and sequential seizures as characteristic seizure types in neonates. A sequential seizure is characterized by clinical manifestation of seizure occurring in sequence (for example, tonic, clonic, myoclonic, and autonomic features following each other without a single predominant feature) with simultaneously ictal pattern change over time according to the clinical manifestation^(4, 16). Considering age of onset and remission, seizure semiology, EEG and imaging findings, the current ILAE classification system defines two epilepsy syndromes in neonates: self-limited neonatal epilepsy (SLNE), where there is likely to be spontaneous remission, and early infantile developmental and epileptic encephalopathy (EIDEE), where there is neurodevelopmental impairment related to both the underlying etiology and to the adverse effects of uncontrolled epileptic activity^(4, 17).

2. *KCNQ2/3* and *SCN2A* genes and epilepsy in newborn

Genetic epilepsy refers to a condition caused by a confirmed or suspected genetic defect where recurrent unprovoked seizures are a primary symptom of the disorder⁽¹⁷⁾. One of the most extensively studied examples to date is genetic neonatal epilepsy linked to genes involved in ion channel regulation, also referred as epileptic channelopathies, such as *KCNQ2*, *KCNQ3* and *SCN2A*, which regulate the function of potassium and sodium channels and play a role in action potential and neuronal firing^(18, 19 20). Mutations in the *KCNQ2* gene encoding the voltage-gated potassium channel Kv 7.2, was the first epilepsy gene to be discovered^(21, 22). The *KCNQ2* gene is located on chromosome 20 and encodes a subunit formed by six transmembrane segments (alpha-helix, S1-S6) and intracellular N- and C-termini. The subunit S4 function as the voltage sensor and S5, S6 and the loop between them forms the ion pore⁽²³⁾. These subunits form tetrameric channels by itself or heteromeric assemblies with the related Kv7.3 subunit encoded by *KCNQ3* gene located on chromosome 8, to generate a slowly-activating, non-inactivating muscarinic acetylcholine receptor modulated current (M-current) in the brain⁽²³⁾. *KCNQ2* and *KCNQ3* are widely expressed, mainly in hippocampus, neocortex and cerebellar cortex⁽²⁴⁾. *SCN2A* gene is located on chromosome 2 and encodes a subunit formed by four domains (I-IV), with each domain containing six transmembrane segments, S1-S6. The S4 segment is the voltage sensor for activation and S5-S6 segments form the ion pore with the selectivity filter⁽²⁵⁾. *SCN2A* is predominantly expressed in cortical excitatory neurons and the cerebellar cortex⁽²⁶⁾.

2.1 - Electroclinical phenotype

2.1.1 - Self limited neonatal epilepsy (SLNE)

In 1998, variants in *KCNQ2* and *KCNQ3* genes were identified as a cause of SLNE^(21, 22, 27), an autosomal dominant epilepsy syndrome characterized by recurrent seizures beginning between day 2 to 8 of life in a previously well neonate and remitting by 16 months of life^(19, 20). Seizure semiology is characterized by focal sequential seizures often beginning with an asymmetric tonic posturing (tonic component) with alternating laterality, accompanied by apnea and desaturations, occasionally progressing to unilateral or bilateral asynchronous clonic component⁽¹⁹⁾. Seizures are brief but can occur up to 30 times a day or even evolve into status epilepticus^(19, 20). Affected neonates display normal interictal EEG, neuroimaging findings and development, although a correlation between seizure frequency and epilepsy later in life was estimated in up to 25%^(19, 28). *KCNQ2* is the primary gene associated with SLNE with mutations identified in over 90% of patients where a genetic cause has been determined⁽²⁰⁾, whereas *KCNQ3* mutations are less frequent⁽²⁷⁾.

In the early 2000s, reports linked mutations in the *SCN2A* gene to the SLNE phenotype^(29, 30). Curiously, it was observed that age of onset varies within families, ranging from the first day of life to 23 months⁽²⁸⁻³¹⁾. Seizures are brief, typically beginning with head and eye deviation and subsequent tonic and clonic movements, staring and apnea. The frequency varies greatly from few episodes without need to treatment to clusters of many per day. Interictal EEG often shows a normal background activity⁽¹⁹⁾. Seizure recurrence is low, with good neurodevelopmental outcome, although cases of episodic ataxia later in life have been reported^(30, 31).

2.1.2 - Early infantile developmental and epileptic encephalopathy (EIDEE)

In 2004 de novo *KCNQ2* variants were identified⁽³²⁾ and rare familial cases with poor outcome were reported⁽³³⁾. In 2012, Weckhuysen et al. identified seven heterozygous missense *KCNQ2* mutations (six de novo, one inherited through paternal mosaicism) in a cohort of epileptic encephalopathy⁽²⁰⁾. Patients affected with *KCNQ2*-related encephalopathy presented the same seizure semiology as in SLNE with onset within the first week of life associated with pharmacoresistant seizures and severe intellectual disability and motor impairment. Their interictal EEG background was abnormal with multifocal epileptiform abnormalities with random attenuation or a burst-suppression pattern and brain MRI showed transient T2 hyperintensities in the globus pallidus⁽²⁰⁾. At the same time, severe phenotypes with encephalopathy in patients with de novo *SCN2A* variants were reported^(34, 35). The most frequent clinical presentation of *SCN2A* is EIDEE with different age at seizure onset presenting either in the neonatal period (neonatal-onset) or in the infantile period (late-onset)^(19, 31). Although seizure semiology shows considerable variation, the predominant seizure types in the neonatal form are focal seizures with tonic component, apnea and a burst-suppression pattern on EEG^(19, 31). Epilepsy is described as pharmacoresistant and patients have severe intellectual disabilities. Later on, abnormalities on brain MRI are frequently reported including cerebral atrophy, thin corpus callosum, T2 hyperintensities in white matter, basal ganglia, thalamus, or brain stem; and moderate cerebellar atrophy^(31, 35).

2.2 - Genetic diagnostic approach

At the same time as cEEG or aEEG is initiated, a comprehensive investigation to identify the underlying causes of seizures is undertaken. Details from family history, as well as antenatal, birth as postnatal history, can assist in determining whether provoking factors or a genetic predisposition are involved⁽⁴⁾. A genetic etiology can be suspected when there is a family history of an autosomal dominant disorder. However, it is important to note that genetic epilepsy does not necessarily equate to being inherited. Significant discoveries have been made regarding the process of inheritance, and an increasing number of de novo pathogenic variants are being identified in both severe and mild genetic epilepsies^(19, 36). To further complicate matters, some patients may be mosaic for a genetic variant^(19, 36). The latest position paper by the ILAE on classification of the epilepsy syndromes in neonates represents an effort to increase awareness of genetic epilepsy⁽⁴⁾ and considering genetic testing as a first-line investigation⁽⁸⁾. The highest diagnostic yield is in early-onset epilepsies⁽³⁷⁾. Different types of genetic testing are available and includes chromosomal microarray, gene panel or exome sequencing⁽¹⁹⁾. Considering epileptic channelopathies, where various genes can be implicated a next generation sequencing gene panel is a good start as it could increase the diagnosis rate and decreased the time until the diagnosis⁽¹⁹⁾. If the panel results are negative a whole exome sequencing (WES) can be performed⁽¹⁹⁾, but with the disadvantage of increasing the detection of variants of unknown significance (VUS). Support for pathogenicity can be drawn from phenotype-genotype correlation, family-segregation analysis or functional validation⁽³⁶⁾. Genetic diagnosis may influence treatment and informed prognosis regarding seizure control and developmental outcome and genetic counselling for families^(19, 36). A key challenge in neonatal epilepsy is that each gene exhibits pleiotropy. *KCNQ2*, *KCNQ3* and *SCN2A* are associated with phenotypes ranging from SLNE to EEDIE which makes precise prognosis challenging for families. This means that careful phenotyping is central to understanding the implications of a genetic result for the patient.

3. Managing seizures in newborn

Considering that up to 50% infants with seizures in the neonatal period exhibit significant sequelae, there is general agreement that seizures require prompt treatment⁽³⁸⁾. In animal models, seizures lead to secondary brain injury and increase the risk of recurrent prolonged seizures if treatment is delayed⁽³⁹⁾. Responsiveness to ASM can diminish with treatment delay of repeated and prolonged seizures⁽³⁸⁾. In human newborns, especially with hypoxic-ischemic brain injury, an association was found between seizure burden and more adverse neurodevelopmental outcomes⁽⁴⁰⁾. Current published guidelines for management of all neonatal seizures recommend initiation of ASM as soon as possible following seizure recognition⁽⁷⁾. Seizures in neonates have been treated as a single entity with protocols that overlooked the role of etiology, managing all cases in the same manner.

Phenobarbital is the most commonly used and studied first-line ASM in more than 90% of neonates with seizures, regardless of etiology, with a variable response rate^(6, 7). The first randomized

controlled trial (RCT) to evaluate the efficacy of first-line ASM for the treatment of seizures in neonates was conducted in 1999 by Painter et al.⁽⁴¹⁾. This study assessed the efficacy of phenobarbital versus phenytoin. Both controlled seizures in less than 50% of neonates with no differences in efficacy. The use of phenytoin as first-line ASM has remained less favored due to the difficulty in maintaining consistent serum concentrations, which arises from its unpredictable absorption and complex pharmacokinetics⁽⁴²⁾. Although, phenobarbital has potential neurotoxic effects and is associated with acute side effects such as respiratory depression, hypotension and sedation and may be associated with lower cognitive scores with long term use^(43, 44), it is the most commonly used ASM for seizures in newborn^(6, 7). In recent years, levetiracetam use has been significantly increased due to its safety profile, the availability of a parenteral formulation and no pro-apoptotic properties were observed on rodent models⁽⁴⁵⁾. Unexpectedly, a second RCT comparing phenobarbital versus levetiracetam showed that phenobarbital was more effective than levetiracetam as first-line ASM⁽⁴⁶⁾.

In 2014, it was shown in a small case series that oxcarbazepine/carbamazepine was effective in seizure freedom in newborns with heterozygous de novo missense pathogenic variants in the *KCNQ2* gene⁽¹⁸⁾. Although carbamazepine has been extensively used in adults, the first reports in neonates date back to 1996, with a study about efficacy and safety of carbamazepine in 10 full term neonates with hypoxic-ischemic encephalopathy⁽⁴⁷⁾. Carbamazepine is a sodium-channel blocker (SCB). Blocking the movement of sodium ions through the channels, carbamazepine limits sustained high-frequency repetitive-firing of sodium dependent action potentials⁽⁴⁸⁾. Besides carbamazepine, other ASM with the same mechanism of action include oxcarbazepine, phenytoin, lidocaine, lamotrigine, lacosamide and zonisamide⁽⁴⁸⁾.

The clinical observation about the efficacy of carbamazepine in patients with *KCNQ2*-DEE was based on the recognition of a distinct electroclinical phenotype characterized by focal tonic seizures affecting alternatively both sides of the body and ictal discharges involving the left or right hemisphere, with a severely abnormal background characterized by multifocal epileptiform abnormalities and random attenuations⁽¹⁸⁾. In a retrospective study of 15 patients with *KCNQ2* encephalopathy, Pisano et al. reported seizure freedom in eight patients on carbamazepine and additional three on phenytoin. It has also been suggested that patients who attained seizure control early tended to have a better outcome⁽⁴⁹⁾. Also, Sands et al. reported evidence that carbamazepine is safe and effective in a cohort of patients with SLNE even in status epilepticus⁽⁵⁰⁾. SCB, in particular phenytoin, have also been proven effective in *SCN2A* neonatal-onset (<3 months) of epilepsy^(31, 51-53).

In the past decade, a potassium channel opener called retigabine/ezogabine that regulates the kv7 potassium channel seemed promising in improving seizure control in patients with *KCNQ2* pathogenic variants, although no patient was reported seizure-free⁽⁵⁴⁾. However, due to its side effects such as blue discoloration of skin and retina, ezogabine was withdrawn from the market⁽⁵⁵⁾.

These studies highlight the importance of considering the etiology of seizure when choosing the first-line ASM and pave the way to early target treatment before genetic results became available. Time to report the genetic results is dependent of local scientific resources and in some countries, they may not be available in a timely manner. The delineation of specific

electroclinical phenotypes will help not only in guiding diagnosis, considering it early in the differential genetic epilepsies, but also to consider a more targeted ASM or gene-based treatment⁽⁵⁶⁾.

4. Outcome predictors

Following the acute stage of seizures in the NICUs, another challenge emerges – predicting the outcome and counselling the family.

The overall prognosis for outcome following seizures in the neonatal period has improved over the past several decades with a significant reduction in mortality⁽⁵⁷⁾. However, the associated comorbidities, such as intellectual disability, cerebral palsy and epilepsy, remain high^(58, 59).

The neuropathological condition responsible for the seizures and EEG variables, namely the background activity and the quantitation of seizure burden, are the most important determinant in predicting neurological outcome⁽⁶⁰⁾.

Normal EEG background activity usually correlates well with outcome, whereas in the presence of abnormal EEG background (burst-suppression, attenuation in voltage or excessive in discontinuity) an unfavorable outcome can be predicted⁽⁶¹⁾.

Although the most of the studies concerning the predictive value of EEG refers to newborns with HIE, EEG background has also been useful in channelopathies, where the course varies from SLNE to devastating EIDEE. In *KCNQ2* well organized interictal brain activity is observed in SLNE whereas in EIDEE a burst-suppression pattern is typically recorded in the first few days of life followed by multifocal epileptiform activity^(19, 20).

A key question is whether seizures adversely affect the newborn brain and thus contribute to a worse outcome. Prior research indicates that seizures occurring during the neonatal period in both humans and experimental animals are linked to disruptions in energy metabolism, alteration of cerebral blood flow and nutrient supply, and to induced neuronal degeneration⁽⁶²⁻⁶⁵⁾. Recurrent or prolonged seizures may contribute to persistent neuronal inflammation, which has been proposed as an important mechanism in the pathogenesis of cerebral palsy^(39, 66).

Recent studies in newborns with HIE using multivariate analyses support the conclusion that higher seizure burden accentuate secondary brain injury and contribute to abnormal outcome^(40, 67).

In *KCNQ2* seizure frequency in the neonatal period has been correlated with epilepsy later in life^(19, 28).

Besides the electroencephalographic aspects in the assessment of outcome, the most important determinant of neurological prognosis is the underlying etiology and when concerning genetic causes for seizures in neonatal period careful information must be provided in addition to seizure control, as the developmental outcomes may be due from the direct effect of the pathogenic variant rather than the effect of the epileptic activity on development^(19, 36).

B - Amplitude-integrated EEG

1. Historical perspectives

The aEEG was devised by Maynard in the late 1960s and its clinical application was developed by Prior to study cerebral activity in adult patients requiring intensive care and undergoing cardiac bypass surgery⁽⁶⁸⁾. This first machine referred to as the cerebral function monitor (CFM), allowed long-term surveillance of brain activity during the time that the patient was exposed to a possible insult, using a simple device that is easy to apply, needs minimal training for interpretation and allows bedside assessment of brain activity^(68, 69). The CFM was later called aEEG to denote a method of electrocortical monitoring and distinguish the method from a specific machine⁽⁷⁰⁾. By the mid-1980s, the first data of newborn infants were published⁽⁷¹⁾. Besides the observation of brain recovery after perinatal asphyxia, these early publications also revealed that electrographic seizures could be recognised in these newborns^(71, 72). Since then, the clinical applications of aEEG have expanded beyond assessment of background activity in asphyxiated term infants to include seizures detection in both term as well as preterm infants, as well as the study of maturational changes and evaluation of sleep-wake cycling (SWC)⁽⁷³⁻⁷⁵⁾. Also, the effects of sedatives, opioids and ASM on the aEEG electrocortical activity have been reported⁽⁷⁶⁻⁷⁸⁾. Although the aEEG has been used in routine clinical brain monitoring and as a research tool in predicting outcome⁽⁷⁹⁻⁸¹⁾, there is an ongoing debate about the value of aEEG in seizure detection⁽⁸²⁾. As noted earlier, aEEG was not designed as a seizure monitor^(68, 69), but the technology has evolved over time and during the early 2000s, modern equipment with digital systems applying the aEEG methodology have emerged. Replacement of paper by digital systems with the possibility of storage and later review and the availability of simultaneous analysis of the raw-EEG from which the aEEG trend is created were indeed a valuable step in improving seizure detection⁽¹⁵⁾. Also, with digital aEEG the use of two channels has become available⁽⁸³⁾. Use of additional channels can improve the sensitivity of seizure detection⁽⁸⁴⁾. In 2020, the ILAE Task Force on Neonatal Seizures recommends "In situations when and where full EEG is not readily available, aEEG may be used with co-registration of raw EEG channels, although its limitations are well recognized"⁽⁴⁾.

2. General principles

2.1 - Signal processing

As originally described by Maynard et al⁽⁶⁸⁾, the EEG signal centered on the alpha band, is first amplified and passed through an asymmetrical band-pass filter that reduces the frequencies to the range between 2 Hz and 15 Hz. This filtering will minimize artifacts from sources such as

sweating, muscle activity, movement, ECG and electrical interference from other equipment used in the NICUs. The filtered signal is then rectified, which means that negative voltages are converted to positive values. After this process, the monitor will display only positive amplitude voltage⁽⁶⁸⁾. Additional processing includes semilogarithmic amplitude compression. The final result is plotted on a scale of amplitudes, that initially runs from 1-10 μ V in a linear manner and from 10-100 μ V in a logarithmic manner to emphasize the changes in the low voltage range and avoiding overloading of the display at high amplitudes⁽⁶⁸⁾. Finally, the aEEG is displayed on highly time compressed (6cm/h) format but with new monitors the speed can usually be customized. Each single vertical line of the aEEG corresponds to 15 seconds of raw EEG signal. The bandwidth of the aEEG reflects the variation in minimum and maximum peak-to-peak amplitudes of the EEG signal⁽⁸⁵⁾. According to Al Naqeeb⁽⁸⁶⁾ a normal amplitude is considered when the upper margin of the aEEG activity was $>10\mu$ V and the lower margin was $>5\mu$ V. Extracerebral artifacts and interelectrode distances can affect the amplitude of the electrocortical activity and lead to incorrect interpretation⁽⁸⁵⁾.

2.2 - Technical aspects

For accurate aEEG tracing, several technical issues must be accomplished⁽⁸⁷⁾:

2.2.1 - Electrode placement

In the original description by Maynard et al.⁽⁶⁸⁾ the recording resulted from the voltage potential difference from left parietal to right parietal (P3-P4 according to the international 10-20 system)⁽⁸⁸⁾. This montage was chosen because it was least affected by muscle and movements artefacts and wouldn't interfere with nursing care. Covering the apex of vascular watershed regions of the brain, P3-P4 montage would be more informative in detecting abnormalities concerning diffuse systemic hypoperfusion. Later on, with the introduction of digital aEEG, the use of two channels has become available⁽⁸³⁾, allowing monitoring beyond the classic biparietal montage. Central and parietal montages (C3-P3; C4-P4) seem to have higher sensitivity than frontal (F3-P3; F4-P4) in detecting seizures⁽⁸⁴⁾. It is very important to respect the interelectrode distance as this can affect the amplitude of electrocortical activity⁽⁸⁵⁾.

2.2.2 - aEEG impedance and electrodes

The impedance is a measure of the quality of the electrode's contact with the skin and scalp. The acceptable range varies from one device to another and is typically found between 0 and 20 kOhm. Impedance values of less than 5 kOhms produce accurate aEEG recordings, although higher values up to 10 kOhms may be allowed in order to avoid excessive manipulation of sensitive skin of the newborn. Marked differences in electrode impedance among electrodes, poor contact, or high impedance should be avoided as this may artificially increase the voltage of the aEEG background⁽⁸⁷⁾. There are various types of electrodes: hydrogel, gold and needle. The ideal electrode would exhibit the minimum possible impedance and it should be as simple as possible to secure and should not easily loosen. More recently, neonatal aEEG caps are an alternative for long-term recording.

2.2.3 - Notch

Notch filter is a valuable signal-processing tool that allows to favor, or pass signals in some frequency band, while markedly attenuating, or rejecting signals in all others.

2.2.4 - Record annotations

The use of annotation during the recording is important and can be done on most aEEG devices with marking an event such as a procedure, medication administration and body movements allowing to identify possible sources of artifacts.

3. Analysis and interpretation

Currently, aEEG and the raw EEG are displayed simultaneously on the monitor screen and thorough examination of the raw EEG should also be part of analysis and interpretation of the aEEG in order to exclude artifacts^(15, 85, 89). As recording proceeds, the aEEG bandwidth created over time, reflects patterns of electrocortical activity, according to gestational age, medication exposure and brain insult. The analysis and interpretation of electrocortical activity in the aEEG is undertaken primarily through pattern recognition that includes classification of background pattern, classification of SWC and presence or absence of seizures⁽⁸⁹⁾:

3.1 - Background pattern

Combining the pattern of the tracings with the amplitude measurements, five background patterns are distinguish according to Hellström-Westas et al⁽⁸⁹⁾ (Figure 1): continuous, which is continuous activity with voltages over 5 μ V in the lower margins and up to 50 μ V in the upper margins; discontinuous, in which there is discontinuous trace with voltages less than 5 μ V in the lower margins and > 10 μ V in the upper margins; burst suppression, periods of very low voltage (inactivity) intermixed with burst of higher amplitude; low voltage, a continuous background pattern of very low voltage (around 5 μ V); flat trace, a very low voltage, mainly isoelectric tracing with activity below 5 μ V.

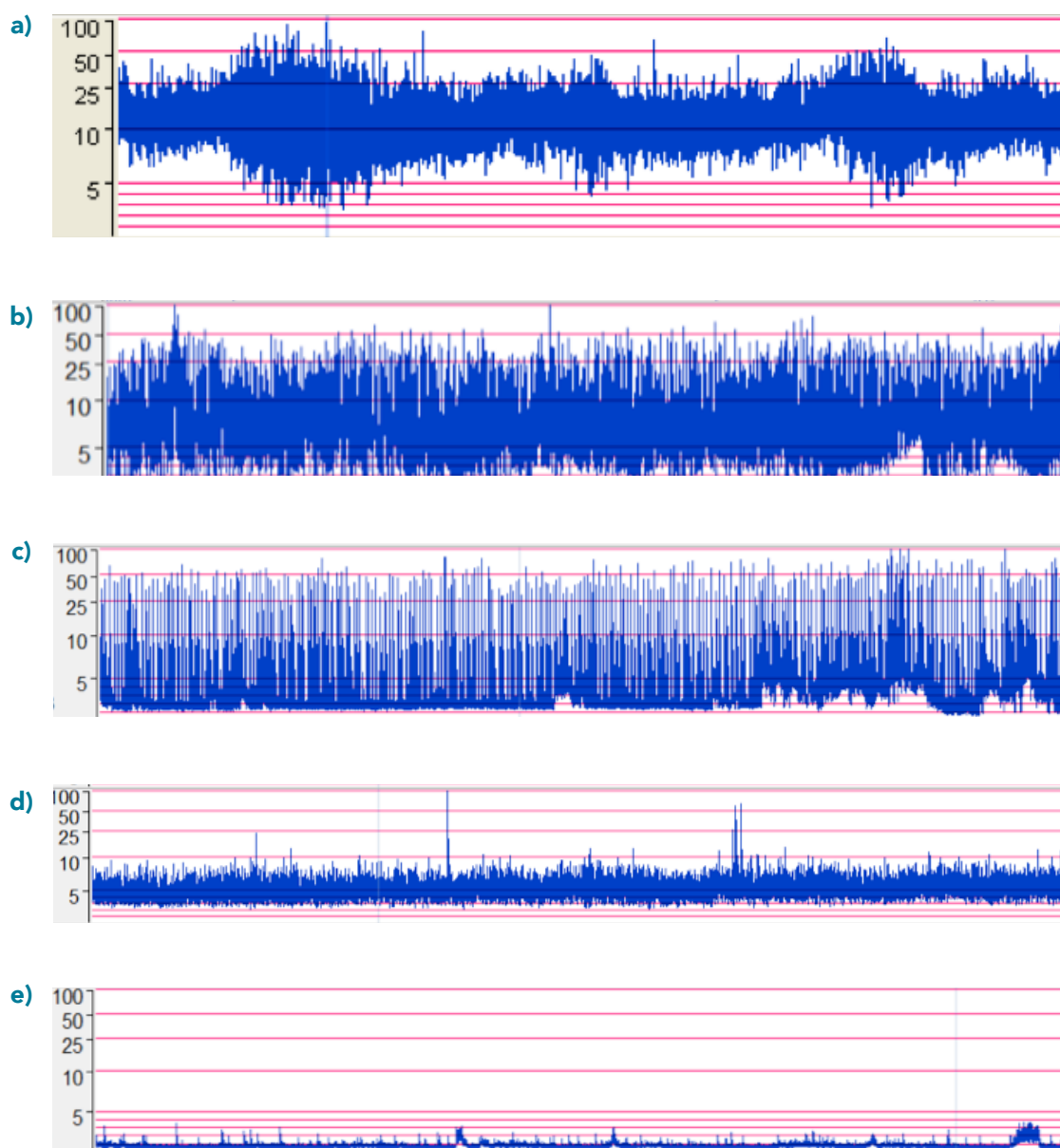


Figure 1. Classification of aEEG background patterns: a) continuous normal voltage with sleep-wake cycling; b) discontinuous; c) burst-suppression, with low burst density; d) low voltage; e) inactive, flat trace.

3.2 - Sleep-wake cycling

Sleep-wake cycling on aEEG is identified by regular changes in bandwidth reflecting the varying peak-to-peak amplitude across different states of wakefulness and sleep^(89, 46, 61). On aEEG periods of wider bandwidth represent discontinuous activity during quiet sleep (QS), whereas periods with a narrow bandwidth represent more continuous activity when awake or in active sleep (AS)⁽⁸⁹⁾. According to pattern recognition, SWC is characterized as periodic changes, mainly of the minimum amplitude and can be classified as absent, immature or developed⁽⁸⁹⁾:

Absent = no cyclic variations in background activity.

Immature = with reference to gestational age, the cyclic fluctuations evident in the minimum amplitude are too infrequent and insufficiently developed.

Developed = clearly identifiable periodic changes between discontinuous and more continuous background activity, with a cycle period of at least 20 minutes.

The presence, time of onset, and quality of sleep-wake cycling is a sign of brain integrity^(74, 90).

3.3 - Electrographic seizures

During an electrical seizure, the overall electro-cortical activity increases which is observed on EEG traces as rhythmic and repetitive spike and wave pattern that gradually builds up and then declines in frequency, morphology or amplitude⁽⁹¹⁾. This peak-to-peak amplitude of an electrographic seizure, that is greater than the amplitude of the interictal background creates an abrupt, transient rise in the aEEG amplitude, maximum and minimum border, occasionally followed by a decrease in aEEG amplitude due to an ictal flattening of the EEG activity^(85, 89). This noticeable rise in the lower and upper aEEG borders can be helpful to identify seizures on the aEEG, irrespective of the underlying etiology and according to their frequency, they can be classified as: single, repetitive or status epilepticus⁽⁸⁹⁾ (Figure 2.):

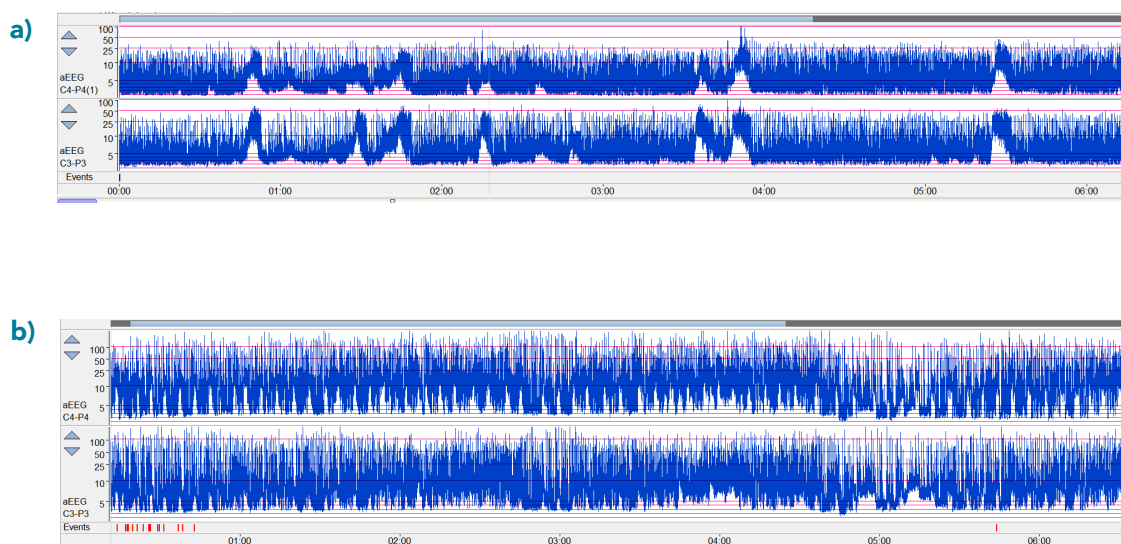


Figure 2. a) Repetitive seizures on a burst suppression background pattern; b) status epilepticus ("saw-tooth pattern") of at least 6 hours duration.

Seizures in the newborn are relatively brief, with an estimated duration of 1-3 minutes in term infants and 2 minutes in preterm infants and are usually well recognized on aEEG⁽¹⁵⁾. However, shorter and low amplitude seizures, may not be detected by the compressed aEEG^(15, 82). When seizures are detected or suspected on aEEG, it is recommended to perform a full EEG, since it remains the gold standard for identifying neonatal seizures⁽⁴⁾. The aEEG was designed to complement cEEG, rather than to substitute it and is intended to be used as a continuous cerebral function monitor providing long-term trends on electrocortical background activity, reflecting patterns that can be easily identified at a glance^(69, 89). The aEEG sensitivity for seizure detection appears higher when⁽¹⁵⁾:

- simultaneous inspection of the raw EEG;
- use of two-channel centrally placed;
- adequate training of the staff;
- use of seizure detection algorithm.

4. aEEG as a prognostic tool

aEEG monitoring is increasingly considered to be the standard of care in the NICUs, not only for detecting seizures but also for monitoring high risk neonates such as preterm, infants suffering from HIE or critically ill infants^(92, 93). Besides its role in seizure detection, the assessment of the background pattern and the presence, quality, and time of onset of SWC has been used to predict outcome in several clinical conditions: HIE⁽⁹⁴⁻⁹⁷⁾, arterial infarction⁽⁹⁸⁾, preterm infants⁽⁹⁹⁾ and newborns with congenital heart disease (CHD)^(100, 101).

Before the use of hypothermia, the prognostic value of early abnormal aEEG background pattern (burst suppression, low voltage, or flat trace) was reported in a meta-analysis⁽⁸¹⁾. A sensitivity of 91% (CI 87%-95%) and a negative likelihood ratio of 0.09 for aEEG tracings was found to accurately predict poor outcome⁽⁸⁰⁾. Continuous recording of aEEG has also been shown to be useful beyond the first 6 hours, especially to evaluate time to recovery of the background activity and to assess time of onset SWC. Newborns who recovered the background pattern within 24 hours after perinatal asphyxia survived without or with a mild disability⁽⁹⁴⁾ and onset of SWC before 36 hours was related with good outcome^(94, 95). Since the widespread use of hypothermia, the predictive value of early aEEG has changed, and cooled infants have been shown to have a normal neurological outcome if the aEEG background activity recovers by 48 h^(96, 97). The absence of SWC has been reported to have prognostic value in term infants with HIE, whether in normothermia^(95, 96) or in cooled infants⁽⁹⁶⁾.

Early prediction of outcome from aEEG is more uncertain in preterm than in full-term asphyxiated newborns. Burst-suppression and low voltage background were associated with poor neurodevelopmental outcome, while normal background and established cyclicality was correlated with a favorable outcome, especially when occurred in the first week of life⁽⁹⁹⁾.

More recently, aEEG has been proven useful on neurodevelopmental outcomes in newborns undergoing cardiac surgery. Delayed recovery in aEEG background was associated in increased risk of early mortality and worse neurodevelopment⁽¹⁰⁰⁾.

2. AIMS

Aims

The general aim of the present thesis is to substantiate the clinical value of aEEG in the diagnosis and management of genetic epilepsies. To achieve this, the study specific objectives include:

1. To assess the ictal aEEG pattern in neonates with *KCNQ2*-related epilepsy;
2. To assess whether other channelopathies (*SCN2A* and *KCNQ3*) show a similar aEEG seizure pattern;
3. To assess whether recognition of the ictal aEEG pattern guided earlier initiation of targeted therapy with carbamazepine or other SCB;
4. To assess whether the aEEG can be useful in outcome prediction of *KCNQ2/3*-related epilepsy;

These objectives were achieved by carrying out a series of studies, whose results are reported in the following chapter:

3. PUBLICATIONS

Article 1

A Distinctive Ictal Amplitude-Integrated Electroencephalography Pattern in Newborns with Neonatal Epilepsy Associated with *KCNQ2* Mutations

Article 2

Early recognition of characteristic conventional and amplitude-integrated EEG patterns of seizures in *SCN2A* and *KCNQ3*-related epilepsy in neonates

Article 3

Distinctive Amplitude-Integrated EEG Ictal Pattern and Targeted Therapy with Carbamazepine in *KCNQ2* and *KCNQ3* Neonatal Epilepsy: A Case Series

Article 1

A Distinctive Ictal Amplitude-Integrated Electroencephalography Pattern in Newborns with Neonatal Epilepsy Associated with *KCNQ2* Mutations

- **Vilan A**, Mendes Ribeiro J, Striano P, Weckhuysen S, Weeke LC, Brilstra E, de Vries LS, Cilio MR. A distinctive ictal amplitude-integrated electroencephalography pattern in newborns with neonatal epilepsy associated with *KCNQ2* mutations. *Neonatology*. 2017;112(4):387-393. Doi:10.1159/000478651.Epub 2017 Sep 20.

A Distinctive Ictal Amplitude-Integrated Electroencephalography Pattern in Newborns with Neonatal Epilepsy Associated with *KCNQ2* Mutations

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Keywords

Neonatal seizures · *KCNQ2* mutations · *KCNQ2*-related epilepsy · Amplitude-integrated electroencephalography · Carbamazepine

Abstract

Background: Recurrent and prolonged seizures are harmful for the developing brain, emphasizing the importance of early seizure recognition and effective therapy. Amplitude-integrated electroencephalography (aEEG) has become a valuable tool to diagnose epileptic seizures, and, in parallel, genetic etiologies are increasingly being recognized, changing the paradigm of the workup and management of neonatal seizures. **Objective:** To report the ictal aEEG pattern in neonates with *KCNQ2*-related epilepsy. **Subjects and Methods:** In this multicenter descriptive study, clinical data and aEEG findings of 9 newborns with *KCNQ2* mutations are reported. **Results:** Refractory seizures occurred in the early neonatal

period with similar seizure type, including tonic features, apnea, and desaturation. A distinct aEEG seizure pattern, consisting of a sudden rise of the lower and upper margin of the aEEG, followed by a marked depression of the aEEG amplitude, was found in 8 of the 9 patients. Prompt recognition of this pattern led to early treatment with carbamazepine in the 2 most recent cases. **Conclusion:** Early recognition of the electroclinical phenotype by using aEEG may direct genetic testing and a precision medicine approach with sodium channel blockers in neonates with *KCNQ2* mutations.

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Introduction

Recognizing seizures in newborns can be challenging as clinical manifestations may be subtle and neonates often exhibit normal motor activity that can be confused with seizures [1]. Conventional bedside electroencepha-

lographic monitoring has greatly improved diagnostic accuracy [2]. Amplitude-integrated electroencephalography (aEEG) is now used routinely in an increasing number of neonatal intensive care units (NICUs) and has proved to be useful for seizure detection [3]. Once seizures are diagnosed, it is important to identify the underlying cause. This can be difficult, given the extensive list of possible etiologies. A stepwise diagnostic approach needs to prioritize the identification of treatable causes [4]. With the recent developments in neuroimaging techniques, the incidence of seizures of unknown etiology has decreased, and genetic defects are increasingly being recognized as an etiology of neonatal seizures [4, 5]. Mutations in *KCNQ2*, encoding the voltage-gated potassium channel Kv7.2, were first described in autosomal dominant benign familial neonatal seizures (BFNS) a self-limiting form of neonatal epilepsy with favorable outcome [6]. Recently, de novo mutations of *KCNQ2* have been reported in children with refractory seizures and poor neurodevelopmental outcome [7–9]. *KCNQ2*-related epilepsies are characterized by recurrent seizures with onset during the first week after birth. Seizure semiology is characterized by asymmetric tonic posturing accompanied by apnea and desaturation, often followed by unilateral or bilateral clonic jerking [10, 11]. Seizures may occur with a frequency of ≥ 10 per day [10, 11]. The interictal EEG is normal in BFNS [10] but may show multifocal epileptiform abnormalities with random attenuations or a burst-suppression pattern in *KCNQ2* encephalopathy [7, 11]. The detection of severely abnormal interictal cEEG background activity is an important feature that helps in discriminating between *KCNQ2*-related encephalopathy and BFNS [10, 11]. A peculiar finding seen in *KCNQ2*-related epilepsies is the marked and postictal generalized EEG attenuation or even suppression (PGES) that occurs even after brief seizures [11, 12]. Brain magnetic resonance imaging (MRI) is normal or shows a transient increase in signal intensity in the globus pallidum [7]. While most antiepileptic drugs (AEDs) have been shown to be relatively ineffective, sodium channel blockers, including oral carbamazepine (CBZ) have been shown to result in prompt seizure control in many cases [10–13], encouraging the use of this drug as a first-line therapy in *KCNQ2*-related epilepsies.

We present the electroclinical data of 9 newborns with refractory seizures due to *KCNQ2*-related epilepsies and describe a distinct ictal aEEG pattern observable in this condition. This highlights the importance of aEEG for early recognition of this genetic diagnosis and prompt personalized treatment of this disorder.

Methods

Nine patients with *KCNQ2*-related epilepsy, admitted between 2003 and 2017 to the NICU of the Hospital São João in Porto, Portugal, and the Wilhelmina Children's Hospital in Utrecht, The Netherlands, were included.

All patients were admitted because of neonatal seizures and were subjected to detailed family history and clinical evaluations. A diagnostic investigation was performed including a workup for infections, a metabolic panel (including values for plasma and cerebrospinal fluid, serum ammonium and lactate, serum amino acids, and urine organic acids), and brain MRI. Continuous aEEG was routinely used soon after admission. In Patients 1, 2, and 3, a single-channel (P3–P4) device was used (Lectromed Devices Ltd., UK; Olympic 6000), and in Patients 4, 5, 6, and 8, a 2-channel (C3–P3; C4–P4) digital device was used (BrainZ or NicoletOne, Natus, Seattle, WA, USA). Patients 7 and 9 were monitored with continuous 8- and 10-channel EEG, respectively (EEG-1200, Nihon Kohden, Tokyo, Japan, and NicoletOne). Standard EEG was performed to obtain additional information. Genomic DNA PCR Sanger sequencing of *KCNQ2* and multiple-dependent probe amplification was performed as previously described [7, 14]. The longest mRNA transcript (NM_172107.2) was used for variant annotation. For variants affecting the protein-coding sequence (nonsense, missense, frameshift, and splice variants), Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>) software was used to determine the in silico likelihood of pathogenicity. Segregation of possible pathogenic variants was analyzed in available family members using Sanger sequencing. The database of the Exome Aggregation Consortium (ExAC, <http://exac.broadinstitute.org>) was used as a control population. The medical records were used for information on neurodevelopmental outcome.

Parental informed consent for inclusion in this study was obtained. The study was approved by the human research ethics committee of the Wilhelmina Children's Hospital.

Results

Clinical and Neuroradiological Features

The clinical and neuroradiological features of the 9 patients are summarized in Table 1. They were all born at term following an uncomplicated pregnancy and with good Apgar scores at delivery. Family histories for epilepsy or neurologic disorders were negative in 8 patients. The mother of Patient 1 had had seizures during the first 3 months of life. Seizure onset in all patients was between the first and twenty-fourth day of life. Seizures were clinically evident in all patients, characterized by asymmetric tonic posturing accompanied by cyanosis, and at times followed by unilateral clonic activity. Laboratory investigation ruled out infections and inborn errors of metabolism namely nonketotic hyperglycinemia, hyperammonemia, urea cycle defect, organic aciduria, hyperlactacidemia, and pyridoxine-dependent and pyridoxalphos-

Table 1. KCNQ2 mutations, aEEG, and clinical features of the 9 patients described in this report

Patient No./ year of birth	1/2003	2/2003	3/2005	4/2006	5/2008	6/2010	7/2014	8/2015	9/2017
KCNQ2 mutation	inherited c.1076C>A p.Thr359Lys	de novo c.1955dupC p.Pro632fs	de novo c.1065C>G p.Asp355Glu	de novo c.2296delC p.Leu766fs	de novo c.1527delA p.Glu509fs	inherited c.1_2_2619+2del (del exon 1–17)	de novo c.830C>T p.Thr277Ile	de novo c.1657C>T p.Arg53Trp	de novo c.901G>A p.Gly301Ser
Mutation previously reported	yes	no	no	no	no	no	yes	yes	yes
Family history	positive	negative	negative	negative	negative	negative	negative	negative	negative
GA/sex	full-term/F	full-term/M	full-term/M	full-term/M	full-term/M	full-term/M	full-term/M	full-term/F	full-term/F
Apgar score	9/10	9/10	9/10	9/10	9/10	9/10	9/10	9/10	9/9
Age at onset and Sz type	1 day, tonic with cyanosis	2 days, tonic with cyanosis	2 days, tonic with cyanosis	3 days, tonic with cyanosis	24 days, tonic with cyanosis	4 days, tonic with cyanosis	2 days, tonic with cyanosis followed by focal clonic activity	1 day, tonic with cyanosis followed by focal clonic activity	1 day, tonic with cyanosis
Ictal aEEG	characteristic	characteristic	characteristic	not characteristic	characteristic	characteristic	characteristic	characteristic	characteristic
Interictal aEEG	CNV	CNV	CNV	DNV	CNV	DNV	DNV	CNV	CNV
AEDs	PB, lidocaine, CZP, pyridoxine, PHT, VPA	PB, lidocaine, MDL, pyridoxine, CZP	PB, MDL, lidocaine, pyridoxine, PHT	PB, MDL, lidocaine, pyridoxine	PB, pyridoxine	PB, MDL	PB, MDL, pyridoxine, LEV, lidocaine, CZP, TPM, VPA	PB, LEV, MDL, pyridoxine, CBZ	PB, lidocaine, MDL, LEV, pyridoxine, CBZ
Response to therapy	lidocaine partially effective	lidocaine partially effective	lidocaine acutely effective, PHT effective	lidocaine partially effective	not effective, but infrequent Sz	not effective, but Sz stopped without further treatment	LEV and lidocaine acutely effective, VPA effective	CBZ effective	lidocaine acutely effective, CBZ effective
Brain MRI	normal	normal	normal	normal	normal	normal	normal	normal	increased signal intensity in GP
Sz outcome	Sz-free at 1.5 months, recurrence of frequent Sz after 4 years	Sz-free at 21 days, recurrence of 2 Sz at 4 years	Sz-free at 12 days, no Sz recurrence	Sz-free at 14 days	Sz-free at 14 days	Sz-free at 18 days, no recurrence	Sz-free at 1.5 months, 1 febrile Sz at 1 year	Sz-free at 15 days, no recurrence	Sz-free at 15 days, no recurrence
Developmental outcome/age at last follow-up	special education, mild intellectual disability, ADHD/ 13 years	mild delay in motor development/ 5 years	normal outcome/ 2 years	lost to FU after discharge	lost to FU after discharge	normal/ 9 months	nonverbal, autistic features, delayed motor development/ 3 years	normal/ 16 months	normal/ 3 months

AEDs, antiepileptic drugs; AS, Apgar score; CBZ, carbamazepine; CNV, continuous normal voltage; CZP, clonazepam; DNV, discontinuous normal voltage; 1, female; FU, follow-up; GA, gestational age; GP, globus pallidus; LEV, levetiracetam; M, male; MDL, midazolam; PB, phenobarbital; PHT, phenytoin; Sz, seizures; TPM, topiramate; VPA, valproic acid.

phate-dependent seizures. Brain MRI was normal in 8 patients. A mild increase in signal intensity in the globus pallidus and a low N-acetyl aspartate level on MRS was seen in 1 patient (Patient 9). Two patients had a few isolated seizures. Six patients had repetitive seizures, 1 of which evolved into status epilepticus (SE). The most frequently used first-line AED was phenobarbital (PB). Pyridoxine was administered to 8 infants without any beneficial effect. All infants needed ≥ 2 AEDs to control their seizures. Lidocaine continuous infusion was trialed in 6/9 infants: 2 were seizure-free during continuous lidocaine infusion and were then switched to oral phenytoin (PHT) or oral CBZ, 1 responded to a combination of lidocaine and levetiracetam (LEV) and was then switched to valproate (VPA), and in 3 patients, lidocaine was only partially effective. The 2 most recent neonates in our series (Patients 8 and 9) were treated with oral CBZ within 1 week after seizure onset, leading to early freedom from seizures. The neurological outcome was highly variable, with a developmental delay evident in 3 patients: patient 1 showed normal language development, mild intellectual disability, and delayed motor development; Patient 2 had a mild delayed motor development; and Patient 7 showed a global developmental delay with absence of language and autistic features. Four patients had a normal outcome, although for 1 of them, follow-up is still limited to 3 months. The remaining 2 patients were lost to follow-up.

Genetic Findings

Genetic findings are detailed in Table 1. The *KCNQ2* mutations included 5 missense mutations, 3 frameshift mutations, and 1 whole-gene deletion (Table 1). Seven mutations occurred de novo and 2 were inherited. Four of the 5 missense mutations have previously been reported in *KCNQ2*-related disorders. Patient 1 was reported previously, and carried a missense mutation inherited from her mother with neonatal seizures [15]. Patients 7 and 9 had de novo missense mutations which have been previously associated with *KCNQ2* encephalopathy [16–18]. Patient 8 had a de novo missense mutation previously reported in both a patient with BFNS and a patient with *KCNQ2* encephalopathy [8, 19]. Frameshift mutations and whole-gene deletions are well-known to be associated with BFNS. One missense mutation was novel (Patient 3) but was predicted to be pathogenic with a high degree of confidence because of its de novo occurrence, its absence in ExAC, its position in a region of the gene with clustering of pathogenic *KCNQ2* variants, and the patient's phenotype.

Amplitude-Integrated EEG Monitoring

Initial aEEG showed a continuous normal voltage in 6 patients and a discontinuous background pattern in 3 (Table 1). A distinct aEEG seizure pattern, consisting of a sudden rise of the lower and upper margins of the aEEG of short duration immediately followed by a marked depression of the aEEG amplitude, was found in 8 of the 9 patients. This was more easily recognized in less time compressed recordings at 15 cm/h (Fig. 1a). Patients 7 and 9 were simultaneously monitored with aEEG and cEEG. The cEEG showed onset of a rhythmic sharpened delta over the right hemisphere during the tonic phase and a prolonged postictal attenuation immediately following the seizure offset (Fig. 1b–d).

Discussion

Neonatal seizures can be challenging in terms of diagnostic workup and management. Etiology of seizures in the neonatal period includes common causes such as hypoxic ischemic encephalopathy, neonatal stroke, intracranial hemorrhage, infectious diseases (meningitis or encephalitis), cerebral dysgenesis, transient metabolic disturbances, and less common, but equally important, causes such as inborn errors of metabolism and genetic epileptic syndromes [20]. Mutations in *KCNQ2*, a gene encoding the voltage-gated potassium channel Kv7.2, were initially discovered in patients with BFNS [6], and later in infants with a neonatal form of epileptic encephalopathy. Mutations in this gene are frequently seen in patients or families with early-onset epilepsy [14] and are estimated to cause between 13 and 23% of all neonatal epileptic encephalopathies [7–9, 11].

All infants in our cohort exhibited the clinical features of *KCNQ2*-related epilepsy. They presented with recurrent focal tonic seizures in the neonatal period. Structural lesions of the brain and infectious and metabolic diseases were excluded in the diagnostic workup.

Continuous aEEG was used in all infants as a standard of care in our NICUs. The aEEG seizure pattern in most common etiologies of neonatal seizures consists of a sudden rise of the lower margin, usually accompanied by a rise in the upper margin of the aEEG, but with no marked depression of the aEEG amplitude at the end of the seizure [21]. The recognition of the same aEEG seizure pattern in 8 of our 9 patients, consisting of an abrupt and short rise of the lower and upper margins followed by a sudden depression of the background pattern due to PGES, does suggest that this is a distinct aEEG seizure pattern. Indeed,

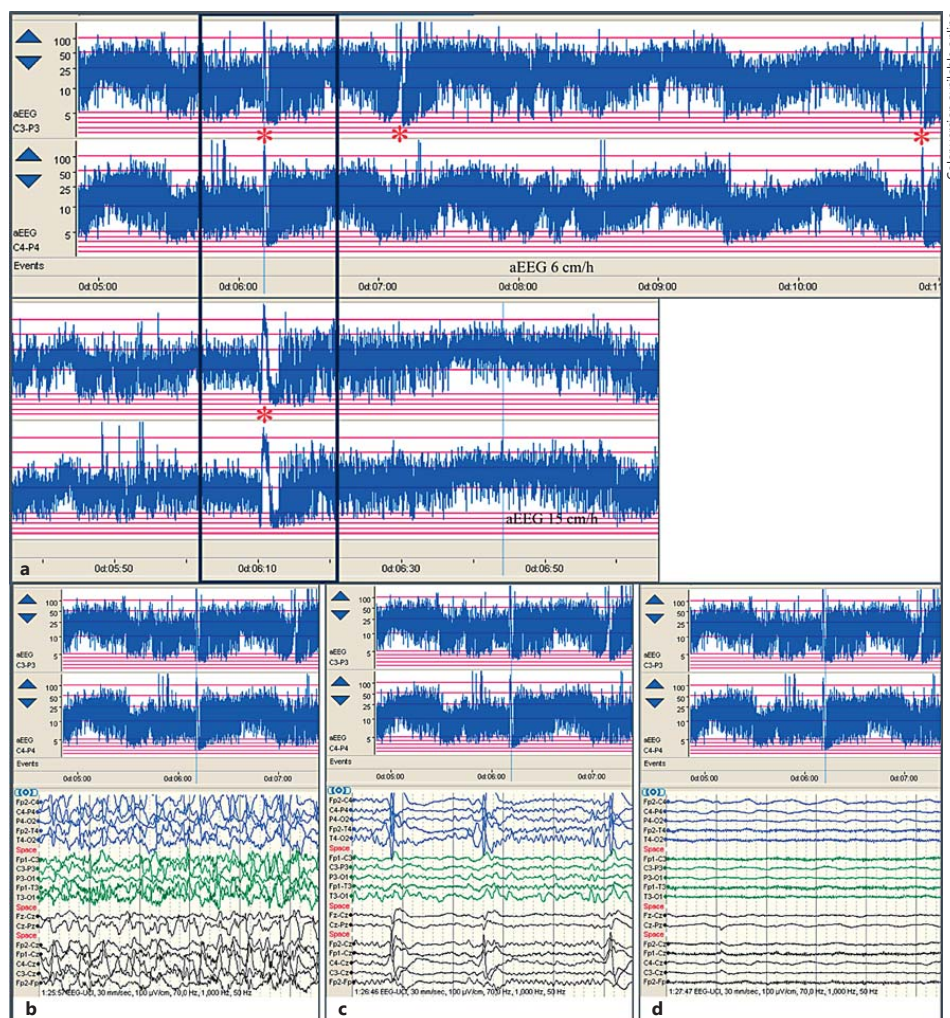


Fig. 1. aEEG/EEG seizure pattern of Patient 7 with KCNQ2-related epilepsy. **a** aEEG at 6 cm/h, shows 3 ictal discharges (asterisks), on a continuous background pattern, identified by brief and subtle rise in amplitude followed by an acute marked reduction of electrocortical activity. This is more easily seen in less time compressed recordings, at 15 cm/h. **b–d** The 6 s of EEG below the aEEG tracing correspond to the dotted vertical lines in the aEEG. The entire seizure is shown in both aEEG and standard EEG.

the marked PGES that was described in the EEG was also present in the aEEG, and it is characteristic of KCNQ2-related epilepsy. Recognition of this distinct aEEG seizure pattern allowed us to identify the KCNQ2-related epilepsy at the bedside in the 2 most recent patients, facilitating early and effective treatment with oral CBZ.

Although cEEG remains the gold standard for the diagnosis of seizures in neonates [3], our findings suggest that aEEG is a useful addition to cEEG for the diagnosis of neonatal epilepsy, especially considering the limited availability of cEEG in many centers, particularly at night and on weekends. In addition, the availability of cEEG is

often limited to standard recordings of 30–90 min, so that ictal events can be missed. aEEG is a useful continuous-monitoring tool based on pattern recognition, and it is easy to interpret at the bedside by neonatologists, but should be complemented by cEEG when possible. Although aEEG tends to have a limited ability to detect focal or short seizures, it proved to be sensitive enough in this study and demonstrated a consistent and easily recognizable pattern in all but 1 of the neonates, allowing for rapid and targeted treatment with CBZ based on electroclinical phenotype alone.

KCNQ2-related epilepsies are characterized by early-onset and, often, frequent, therapy-resistant seizures [10–12]. All infants in our cohort failed to respond to multiple AEDs. PB, the first-line treatment for seizures in the neonate [22], was not effective in our series, and multiple drugs were administered in order to obtain seizure control. Reid et al. [23] describe a positive response of seizures to vitamin B₆ in patients with KCNQ2-associated epilepsy. All but 1 of our patients were trialed with an adequate dose of pyridoxine, and although a transitory effect was noted in some, seizures recurred in all. In our series, lidocaine was at least partially effective in 6 of the patients to whom it was administered. All infants treated with oral CBZ promptly responded with sustained freedom from seizures. AEDs acting on sodium channels, including CBZ and PHT, were found to be safe and effective in controlling seizures in KCNQ2-related epilepsy, and are now considered a first-line treatment [11–13]. While lidocaine is also a sodium-channel blocker, concerns about cardiac toxicity have limited its use in the NICU, even though a recent study showed the drug to be effective and safe in a large number of newborns [24]. CBZ is rarely used in the NICU [25, 26]. However, oral CBZ has recently been reported to be a safe and effective AED to control seizures in infants with KCNQ2-related epilepsy, both BFNS and KCNQ2 encephalopathy [10, 11, 13], and sodium channel blockers are now recommended as established precision medicine treatment in these disorders [27]. In patients 8 and 9, the most recent patients, CBZ was initiated as soon as KCNQ2-related epilepsy was suspected (based on the electroclinical phenotype consisting of focal tonic seizures and the reported aEEG pattern), and a few days later, the infants were seizure-free and remain so at the time of this report.

All infants had pathogenic *KCNQ2* variants, either novel or previously identified in patients with KCNQ2-related epilepsy. Interestingly, the variant found in patient 8 was previously reported in a patient with intellectual disability as well as in a patient with normal develop-

mental outcome, suggesting that several factors may determine the severity of the phenotype in patients with an identical variant. Specifically, as of yet unknown genetic modifiers may play a major role in modulating the phenotypic counterpart of a given genetic mutation [28]. In addition, given the evidence from both animal and human studies that recurrent seizures in an immature brain may interfere with normal brain development and contribute to long-term neurological disability [29, 30], it is possible that the early seizure control contributed to a more favorable disease course.

In the last few years, we have seen great advances in the understanding of genetic epilepsies that present with seizures in the neonatal period. Once acute symptomatic etiologies, including hypoxic ischemic encephalopathy, stroke, and infections, have been excluded, a genetic etiology should be considered. Knowledge of the electroclinical features including alternating tonic seizures associated with typical aEEG correlates is a valuable tool for the recognition of KCNQ2-related epilepsy in the NICU, and a consideration for the early use of sodium channel blockers while waiting for the genetic confirmation. The importance of early diagnosis will be crucial in future targeted treatment trials that aim to not only eliminate seizures, but also change the developmental prognosis of genetic epilepsies, as a treatment may be more effective if applied in the early stage of the disease.

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Disclosure Statement

The authors declare no conflicts of interest.

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Article 2

Early recognition of characteristic conventional and amplitude-integrated EEG patterns of seizures in *SCN2A* and *KCNQ3*-related epilepsy in neonates

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Early recognition of characteristic conventional and amplitude-integrated EEG patterns of seizures in SCN2A and KCNQ3-related epilepsy in neonates

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ABSTRACT

Purpose: Early recognition of seizures in neonates secondary to pathogenic variants in potassium or sodium channel coding genes is crucial, as these seizures are often resistant to commonly used anti-seizure medications but respond well to sodium channel blockers. Recently, a characteristic ictal amplitude-integrated electroencephalogram (aEEG) pattern was described in neonates with KCNQ2-related epilepsy. We report a similar aEEG pattern in seizures caused by SCN2A- and KCNQ3-pathogenic variants, as well as conventional EEG (cEEG) descriptions.

Methods: International multicentre descriptive study, reporting clinical characteristics, aEEG and cEEG findings of 13 neonates with seizures due to pathogenic SCN2A- and KCNQ3-variants. As a comparison group, aEEGs and cEEGs of neonates with seizures due to hypoxic-ischemic encephalopathy ($n = 117$) and other confirmed genetic causes affecting channel function ($n = 55$) were reviewed.

Results: In 12 out of 13 patients, the aEEG showed a characteristic sequence of brief onset with a decrease, followed by a quick rise, and then postictal amplitude attenuation. This pattern correlated with bilateral EEG onset attenuation, followed by rhythmic discharges ending in several seconds of post-ictal amplitude suppression. Apart from patients with KCNQ2-related epilepsy, none of the patients in the comparison groups had a similar aEEG or cEEG pattern.

Discussion: Seizures in SCN2A- and KCNQ3-related epilepsy in neonates can usually be recognized by a characteristic ictal aEEG pattern, previously reported only in KCNQ2-related epilepsy, extending this unique feature to other channelopathies. Awareness of this pattern facilitates the prompt initiation of precision treatment with sodium channel blockers even before genetic results are available.

1. Introduction

In neonates, most seizures are attributable to acquired non-genetic causes, including hypoxic-ischemic encephalopathy (HIE), vascular

events or infectious diseases. A considerable subgroup, however, has a genetic basis. Many epilepsy-related genes encode ion channels, and epilepsies caused by pathogenic variants in this group are often referred to as channelopathies [1–3]. Many of the channelopathies-related

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epilepsies are associated with defects of voltage-gated sodium, potassium or calcium channels. Neonatal-onset epilepsies due to channelopathies are most frequently caused by mutations in the *KCNQ2* and *SCN2A* genes, and to a lesser extent in the *KCNQ3* gene [4–6]. Variants in these genes are now known to be associated with a spectrum of phenotypes, ranging from self-limited (familial) neonatal epilepsy to severe neonatal epilepsy syndromes, including developmental and epileptic encephalopathy (DEE) [7,8]. Accordingly, the prognosis regarding seizure control also comprises a wide spectrum, from self-limited or well-controlled to drug-resistant seizures [9].

Seizures associated with these channelopathies are typically less responsive to common first choice anti-seizure medications (ASMs), such as phenobarbital and benzodiazepines. However, the administration of sodium channel blockers, including oxcarbazepine, carbamazepine, phenytoin, and lidocaine can provide seizure freedom or a significant reduction in the majority of neonates [8,10]. As a lower seizure burden may be associated with better developmental outcome, regardless of the etiology or severity of neurological illness [11], a sodium channel blocker should be initiated as soon as possible when a channelopathy, particularly due to *KCNQ2*, *KCNQ3*, or *SCN2A* gene defect, is suspected [8,10,12]. This emphasizes the critical need for early recognition of this disorder to ensure adequate early seizure control in both the self-limited and severe phenotypes.

Recognition of *KCNQ2*, *KCNQ3* and *SCN2A*-associated epilepsy can be based on seizure semiology, typically comprising asymmetric tonic posturing, associated apnea and desaturation, often followed by unilateral or bilateral asynchronous clonic jerks [7,8]. However, distinction with seizures due to other aetiologies solely based on clinical semiology may be difficult. Recently, seizures in *KCNQ2*-related epilepsy in neonates were shown to have a characteristic pattern on the amplitude-integrated EEG (aEEG) with a sudden rise in amplitude, followed by a marked prolonged postictal attenuation [13]. This unique aEEG pattern can be quickly recognised by neonatologists at the bedside [13]. In our descriptive study of thirteen newborns with *SCN2A* and *KCNQ3*-related epilepsy, we demonstrate a similar pattern on the aEEG and cEEG, extending this unique feature to other channelopathies. These features, in combination with clinical semiology may allow for early recognition, not only by the bedside team but also by the neurology, neurophysiology, and genetic team.

2. Methods

This is a retrospective, descriptive case series of 13 neonates with seizures secondary to confirmed (likely) pathogenic *SCN2A* or *KCNQ3* variants from five different centers (Leiden University Medical Center, the Netherlands (LUMC); Erasmus Medical Center, the Netherlands (EMC); Alberta Children's Hospital in Calgary, Canada (ACH); Benioff Children's Hospital, University of California, San Francisco (USCF); Hospital São João in Porto, Portugal (HSJ)). Patients were included consecutively in each center. All patients were admitted to their respective Neonatal Intensive Care Units (NICUs) because of seizure onset between January 1, 2013 and October 31, 2021. All individuals identified within this time frame at the participating institutions had been offered standard of care clinical genetic testing in case of unknown etiology of seizures, and all individuals with a likely pathogenic or pathogenic variant in *SCN2A* and *KCNQ3* were included. In each center, a complete clinical assessment was performed, including detailed physical examination and family history. Extensive diagnostic work-up, including a brain MRI, investigations for infections (blood, urine and CSF culture), and metabolic disorders (including CSF, blood and urine analysis) were performed in all patients. Only patient 12 did not have a brain MRI. Continuous brain function monitoring was carried out as per local protocols in each center. At LUMC, continuous aEEG monitoring was performed routinely, using a 2-channel digital device (F3-P3, F4-P4) in patients 1–3 (NicoletOne, Natus System, Pleasanton, CA, USA). Restricted 10–20 system bipolar EEG, including eleven electrodes Fp1,

Fp2, T3, T4, T7, T8, C3, C4, Cz, O1, O2, was completed for at least one hour in all patients using EEG-1200, Nihon Kohden, Tokyo, Japan. At EMC, patients 4 and 5 were continuously monitored with a 2-channel (F3-P3, F4-P4) aEEG monitor (Brainz BRM3 Brain Monitor, Natus System, San Carlos, CA, USA). At ACH, patients 6–8 and 10–11 were continuously monitored with conventional 11-electrodes restricted 10–20 neonatal EEG montage including the following leads Fp1, Fp2, C3, C4, T7, T8, O1, O2, Pz, Cz, Fz (NeuroWorks – Natus System. Persyst 12 software. Natus, Pleasanton, CA, USA). At UCSF neonates were monitored with continuous video-EEG using a Nicolet One vEEG system (patient 9) or Natus Networks system (patient 12); both using a total of nine electrodes including Fp1, Fp2, C3, C4, T7, T8, O1, O2, and Cz. At HSJ, patient 13 was continuously monitored with a 2-channel digital device (F3 - P3, F4 - P4) (NicoletOne, Natus System, Pleasanton, CA, USA). Rapid trio whole exome sequencing or comprehensive epilepsy gene panel diagnostics was performed in all patients via clinical laboratories. Where possible, variants were clinically segregated in parents to determine inheritance. All variants were reviewed for pathogenicity according to ACMG criteria [14].

To assess whether the described (a)EEG findings are specific for *KCNQ2*-, *KCNQ3*- and *SCN2A*-related epilepsy, aEEGs and cEEGs of two comparison groups were reviewed, all patients meeting criteria within the defined time frame: i) neonates with seizures due to HIE, treated with therapeutic hypothermia and admitted at the NICU of ACH between April 1, 2015 and October 31, 2020, and ii) patients with neonatal seizures due to confirmed likely pathogenic or pathogenic genetic variant affecting channel function and associated with epilepsy, admitted at the NICU of ACH in Calgary between January 1, 2012 and March 31, 2023.

Parental informed consent was obtained and signed for all patients included in the case series. As individual data is not shown for the comparison groups, parental informed consent was not required. Research Ethics Board approval was obtained in Calgary for this collaborative research (#REB16–1783).

3. Results

3.1. Clinical and neuroradiological features

Thirteen neonates, 6 male and 7 female, were included in this study. Clinical, aEEG, EEG and neuroradiological features are summarised in Table 1. All patients were born at term or late preterm, following an uncomplicated pregnancy and delivery. Family history for seizures was negative in 9 patients. Seizure onset was at day 1–3 for the 9 neonates with *SCN2A* variants (patients 1–9) and at day 1–6 for the 4 neonates with *KCNQ3* variants (patients 10–13). Interictal neurological examination was normal in 8 patients but demonstrated mild to severe axial hypotonia and decreased spontaneous movements in 5 patients, all with *SCN2A* variants. Seizure semiology typically included asymmetric tonic posturing (all patients), accompanied by apnea, desaturation or cyanosis (9/13 patients). Other observed features, usually preceding or following the tonic posturing, included stereotypic behavior, head deviation, eye movements or staring.

Infections and inborn errors of metabolism were excluded by laboratory testing in all patients, and MRI was normal in most patients (8/12 patients). In four patients, all with *SCN2A* variants, MRI showed restricted diffusion in the medial thalamus the right caudate nucleus, periventricular white matter abnormalities or a thin corpus callosum (Table 1).

In all patients, various ASMs were trialed before adequate seizure control was reached (median number of used ASM 4; range 1–6). In 12 out of 13 patients, a sodium channel blocker was the last type of ASM introduced, achieving significant seizure reduction in all patients: nine patients were seizure free upon an adequate dosage, and three patients had a partial reduction in seizures (>50% reduction in the number of seizures). One patient became seizure-free on phenobarbitone. In seven

Table 1
Clinical characteristics of 13 patients with neonatal-onset epilepsy related to SCN2A and KCNQ3 mutations.

Patient No.	1 (NLL1)	2 (NLL2)	3 (NLL3)	4 (NLR1)	5 (NLR2)	6 (CAA1)	7 (CAA2)	8 (CAA6)	9 (USF2)	10 (CAA4)	11 (CAA5)	12 (USF1)	13 (POP1)
Mutation	SCN2A de novo c.4644G>C, p. (Met1548Ile)	SCN2A de novo c.2306T>C, p. (Ile769Thr)	SCN2A de novo c.5528A>T, p. (Asp1843Val)	SCN2A de novo c.4061T>C, p. (Met1354Thr)	SCN2A unknown c.3967A>G, p. (Met1323Val)	SCN2A de novo mosaic for c.2635G>A, p. (Gly879Arg)	SCN2A unknown c.4886G>A, p. (Val261Met)	SCN2A de novo c.781G>A, p. (Val261Met)	SCN2A de novo c.2713A>G, p. Ly905GluE	KCNQ3 de novo c.1558C>T, p. (Arg520*)	KCNQ3 paternally inherited, c.938C>T, p. Thr313Ile	KCNQ3, paternally inherited c.923 G>C, (Tyr319Cys)	KCNQ3 de novo c.956A>G, p. (Tyr319Cys)
Family history	negative	negative	negative	negative	negative	negative	negative	negative	negative	positive, possible paternal history of seizure	positive, father positive, had childhood febrile seizures in mother	negative	negative
GA, sex	full-term/M	full-term/F	full-term/M	full-term/F	full-term/M	full-term/F	full-term/M	full-term/M	36w4d/F	full-term/M	full-term/F	full-term/F	full-term/F
Age at onset	9,10	8,8	9,10	9,10	9,10	9,9	5,9	9,9	9,9	7,8	8,9	8,9	9,10
Sz type	normal	restricted diffusion medial left thalamus	small periventricular white matter lesion	normal	normal	restricted diffusion right caudate nucleus	normal	normal	thin corpus callosum	normal	normal	not performed	normal
Age at onset Sz type	day 2 multifocal tonic or tonic-clonic or stereotypic behavior	day 2 multifocal tonic, desaturation	day 2 focal tonic, desaturation, stereotypic behavior, head deviation and clonic movements	day 2 generalised clonic and apnea	day 3 generalised tonic head deviation, yawning, and apnea at the end a few clonic movements	day 1 generalised tonic posturing, movement of the eyes, desaturation with cyanosis, followed by posturing	day 3 apnea, desaturation and hypotonic; focal tonic or tonic – clonic followed by posturing	day 2 unilateral focal tonic or tonic- clonic, then, sequential whole body tonic posturing	day 1 eye deviation, sometimes followed by tonic extension of the extremities and brief self- resolving desaturation	day 1 tonic-clonic	day 6 opening eyes, staring, apnea with cyanosis, focal tonic posturing to bilateral tonic, clonic jerks desaturation	day 4 day 3 tonic phase posturing with cyanosis	day 3 tonic posturing with cyanosis
Interictal neurological examination	encephalopathy/axial and limb hypotonia with alternating opisthotonus	axial hypotonia	axial hypotonia	normal	normal	axial and limb hypotonia proximal > distal	normal	Normal, mild encephalopathy due to med loads	axial hypotonia	normal	normal	normal	normal
Ictal aEEG Interictal aEEG background pattern	characteristic ¹ BS	characteristic ¹ CNV	characteristic ¹ CNV	characteristic ¹ CNV	characteristic ¹ CNV	characteristic ¹ CNV	non- characteristic CNV	characteristic ¹ CNV	characteristic ¹ BS	characteristic ¹ CNV	characteristic ¹ CNV	characteristic ¹ CNV	characteristic ¹ CNV
ASMs in neonatal period	PB, PN, LVT, OXC	PB, PN, LVT, lidocaine, OXC	PB, LVT, OXC	PB, CBZ	PB, CBZ, LEV, MDZ, OXC, PHT	PB, LVT, TPM, PHT, PB, PN, CBZ	PB, LVT, OXC	PB, LVT, PHT, CBZ	PB, LVT, PN, CLB, VGB, CBZ, PPHt,	PB, CBM, LVT, LVT, PB	PB, CBZ	PB, CBZ	PB, CBZ
Effective ASM (dosage)	PB partially effective (7.5 mg/kg in 2 doses, after loading doses) OXC effective (30 mg/kg in 2 doses)	OXC effective (30 mg/kg in 2 doses)	OXC effective (30 mg/kg in 2 doses)	CBZ effective (7 mg/kg in 2 doses)	CBZ (20 mg/kg in 2 doses), OXC (35 mg/ kg in 2 doses) and PHT (13 mg/kg in 2 doses after loading doses) all partially effective	CBZ partially effective (30 mg/kg in 3 doses + PPHt in 2 doses)	OXC effective (18 mg/kg in 2 doses)	PHT (20 mg/kg in load), CBZ (10 mg/kg/day)	CBZ partially effective (40 mg/kg/day)	PB and load PPHT effective (PPHT load 10 mg/kg, maintenance PB 5 mg/kg/ day in 2 doses)	PB effective (load 20 mg/ kg; maintenance 5 mg/kg/day in 2 doses)	CBZ effective (10 mg/kg/d)	CBZ effective (15 mg/kg in 3 doses)
Serum concentrations	OXC <0.5 mg/l; 10- OH-CBZ 11 mg/l (NV 10–35 mg/l, day 30, dosage 31 mg/kg/ mg/l (NV	OXC undetectable; 10-OH-CBZ 6 mg/l (NV	unknown	unknown	CBZ 2.3 mg/l (NV 4–12, dosage 20 mg/ kg/day)	CBZ 79 umol/L (NV <50 umol/L, dosage 30 mg/ kg/day)	unknown (not performed due to low maintenance	unknown	unknown	unknown	PB 125 umol/L (NV 65–170 umol/L, day 9, dosage	unknown ml, day 9, dosage	CBZ 5.77 ug/ ml (4–12 ug/ ml, day 9, dosage

(continued on next page)

Table 1 (continued)

Patient No.	1 (NLL1)	2 (NLL2)	3. (NLL3)	4 (NLR1)	5 (NLR2)	6 (CAA1)	7 (CAA2)	8 (CAA6)	9 (USF2)	10 (CAA4)	11 (CAA5)	12 (USF1)	13 (POP1)
	day)	10–35 mg/L day 3, dosage 10 mg/kg/day dosage) OXC <0.5; 10- OH—CBZ 24 (day 11, dosage 30 mg/kg/day)	sz-free (day 19)	sz-free (day 10)	sz-free (day 3)	Almost sz-free (14 months)	sz-free (6 weeks) with CBD oil (5 y.o.)	sz-free (day 14) dose)	several seizures per week (n.a.)	sz-free (day 7)	sz-free (day 8)	Sz-free (day 4)	sz-free (day 8)
Sz outcome (offset age)	sz-free (day 18)	sz-free (day 19)	sz-free (day 10)	sz-free (day 3)	Almost sz-free (14 months)	sz-free (6 weeks) with CBD oil (5 y.o.)	sz-free (day 14) dose)	several seizures per week (n.a.)	sz-free (day 7)	sz-free (day 8)	Sz-free (day 4)	sz-free (day 8)	sz-free (day 8)
Time between initiation effective ASM and Sz outcome	6 days	11 days	2 days	<24 h	4,5 weeks	<24 h	<48h	1 day	n/a	<24h	<24h	1 day	1 day
Duration of hospitalization	28 days	29 days	12 days	4 days	11 weeks	21 days	4 days	21 days	5 weeks	3 days	3 days	4 days	6 days
Duration of initial ASM therapy	2 years 4 months	1 year 4 months	6 months	9 months	ongoing (OXO)	ongoing	ongoing	1 year 6 months	ongoing	ongoing (switch to CBM, week 4). Last Sz 4 mo prior to last F/ U	12 months	1 year	ongoing
Recurrence upon initial seizure-free state (age)	yes SWAS (4 years)	yes two unprovoked seizures (2 years)	no only provoked seizures	yes (day 14, addition CBM)	n/a	Evolved into epilepsy of infancy with migrating focal seizures (day 10). Controlled day, free at 4 y.o. w/CBD oil.	yes (6 months, OXC dose adjusted to 30 mg/kg/ 10). Controlled day, free at 4 y.o. w/CBD oil.	No	n/a	yes (4, 6, and 20 months)	no	no	no
Recurrence upon discontinuation of ASM therapy	yes	yes	no	no	n/a	2 weeks after D/C of CBD oil. Controlled again w/CBD oil	n/a	No	n/a	n/a	no	no	n/a
Developmental outcome (Last FU; age)	severe developmental delay (6 years)	Very mild developmental delay (5 years)	normal development (3 years)	Severe developmental delay, hypotonia (2 years)	Moderate impaired motor development (1 year 2 months)	severe impaired motor and speech development (5 years 1 month). GMFCS 5	expressive- receptive language, mild gross motor. (1 year 2 months)	normal (3 years 4 months)	severe intellectual disability, non- verbal, non- ambulant (7 years)	normal development (4 years)	normal development (4 years)	normal development (1 year 8 months)	normal development (1 year 8 months)

¹ Characteristic means the typical pattern of a sudden attenuation, then rise in amplitude, followed by a marked postictal suppression, as described in Fig. 1. ASMs anti-seizure medications; BS burst suppression; CAA Canada Calgary; CBZ carbamazepine; CBM Clobazam; CNV continuous normal voltage; DNV discontinuous normal voltage; SWAS spike-wave activation in sleep; F female; FPHT fosphenytoin; FU follow-up; GA gestational age; LVT levetiracetam; M male; MDZ midazolam; n/a not applicable; NLL Netherlands Leiden; NLR Netherlands Rotterdam; NV normal value; OXC oxcarbazepine; PB phenobarbitone; POP Portugal Porto; PHT: phenytoin; PN pyridoxine; sz: seizure; TPM topiramate; VGB vigabatrin. All SCN2A variants reported on transcript NM_021007.3. All KCNQ3 variants reported on transcript NM_004519.3.

patients (3–5, 7–8, 12–13), a sodium channel blocker was the second or third line ASM, when the treating physicians recognised the unique aEEG pattern suspecting a channelopathy. Median follow-up was over 3 years (range 14 months – 7 years). One child (patient 6) developed epilepsy of infancy with migrating focal seizures at day 10. In seven patients the ASM was discontinued (after maintenance therapy of 6 months – 2.5 years), upon which 5 children remained seizure free. One of the children with epilepsy recurrence (patient 1) developed SWAS after 4 years of age, responding well to oral prednisolone treatment. Development ranges from normal to severe developmental delay (Table 1). All 4 individuals with *KCNQ3* variants had a normal outcome.

Regarding the comparison groups, 123 patients with HIE and hypothermia protocol were identified, of which 117 patients had aEEG and cEEG monitoring. Out of these 117 patients, 82 patients had seizures during EEG monitoring: either electroclinical seizures ($n = 55$), and / or electrical seizures without clinical correlate ($n = 75$). As for the comparison group of patients with a confirmed genetic cause affecting channel function and associated with epilepsy, 55 patients were identified. Out of these 55 patients, 11 patients had seizures in the neonatal period. In these patients, (likely) pathogenic variants were found in the *KCNQ2* ($n = 6$), *ATP1A3* ($n = 1$), *CACNA1A* ($n = 1$), *GABRB3* ($n = 1$), *KCNT1* ($n = 1$), *SCN8A* ($n = 1$) genes.

3.2. Genetic findings

All variants found in the *SCN2A* or *KCNQ3* genes were pathogenic or likely pathogenic according to the ACMG classification [14]. For patient 10, the *KCNQ3* variant was interpreted as likely pathogenic but this was in the context of recessive disease and it is unclear in the context of dominant disease. We identified six new protein variants that were not previously published (Supplementary Table S1). An individual with a variant affecting the same residue as patient 4 and 9 has been previously published [15,16], and functional studies and characterization of epilepsy in patient 11 were recently reported [17,15]. Furthermore, patients 6–8 and 12 have been previously published [18–23].

3.3. aEEG and EEG findings

The ictal recording showed a characteristic pattern in the aEEG in all except one patient (patient 7). This typical pattern is shown for a patient with *SCN2A*- (Fig. 1A) and *KCNQ3*-related epilepsy (Fig. 1B). On the aEEG, a seizure can be recognized by a brief decrease in the upper margin, then a sudden rise in both the lower and the upper margin, followed by the characteristic marked postictal attenuation (decreased of both margins). When increasing the speed of the aEEG (from 6 to 15 cm/h), the pattern may become even more obvious. This pattern is different from aEEG findings in symptomatic seizures, in which a gradual rise of the upper and lower margin is seen at onset with usually longer duration without postictal attenuation (Supplementary Fig. S1).

Interictally, most patients (11 out of 13) had a continuous normal voltage background on their aEEG, with 1 patient showing intermittent episodes of suppression lasting 3–4 s.

The typical ictal aEEG pattern correlates to findings on the cEEG shown in Fig. 2, and Supplementary video 1. At the onset of seizures, a desynchronised diffuse background attenuation is seen (corresponding to the lowering of the upper margin in the aEEG), followed by gradual amplitude increase of rhythmic discharges (seen as both upper and lower margin increases on aEEG) and a post-ictal attenuation-suppression (correlating to the attenuation on the aEEG, both margins being low). For patient 7, there was a single recorded seizure with a partially similar EEG pattern of focal attenuation onset and, 5 s later, being followed by diffused 2 Hz delta rhythmic discharges. This was followed by minimal postictal suppression. Once medicated, his seizure pattern changed to a more diffused theta rhythmic activity without onset attenuation or post-ictal suppression.

Interictally, the EEG may range from almost normal (Fig. 3A), to a

typical sustained suppression-burst pattern (Fig. 3B).

In the comparison group of neonates with HIE, none of the patients had a similar pattern on aEEG or cEEG. Likewise, none of the patients in the genetic channelopathy comparison group, apart from patients with *KCNQ2*-associated epilepsy had the above described pattern.

4. Discussion

We describe 13 patients with neonatal-onset seizures caused by pathogenic or likely pathogenic variants in either the *SCN2A* or *KCNQ3* genes, and demonstrate that seizures due to these two channelopathies are typically, at some point, characterized by a specific pattern on aEEG and cEEG. This typical ictal pattern consists of brief onset with an initial decrease, and then a quick rise in aEEG amplitude, followed by postictal attenuation. This correlates to a period of bilateral attenuation on the conventional EEG at onset, followed by rhythmic focal or bilateral discharges ending in post-ictal amplitude suppression for several seconds. These findings are similar to the ictal pattern described in patients with *KCNQ2*-related epilepsies [13]. Apart from patients with *KCNQ2*-related epilepsy, none of the patients with seizures in the neonatal period with other genetic channelopathies in our comparison group had a similar pattern on aEEG or cEEG.

In combination with the seizure semiology, this characteristic ictal pattern on aEEG and cEEG should raise high suspicion for a *KCNQ2*-, *KCNQ3* or *SCN2A*-related epilepsy. However, since not all patients have this specific aEEG or cEEG pattern, the absence of the ictal EEG pattern, does not rule out these channelopathies. In our study, some patients had focal seizures at onset but only at a later time developed the characteristic pattern described here (i.e. patient 8). While we cannot fully exclude the possibility of missed cases of *KCNQ2*-, *KCNQ3* or *SCN2A* channelopathy, either with or without the characteristic aEEG pattern, we suspect selection bias for this study is low. Standard of care at the participating institutions during the indicated time frame would have included EEG monitoring and all individuals with unknown etiology of seizures would have been offered genetic testing for these genes. All individuals with neonatal seizures that had genetic testing identifying a relevant variant would have been identified.

Seizures associated with channelopathies are often resistant to commonly used ASM, but do respond well to sodium channel blockers [8,10,24]. Therefore, the characteristic ictal aEEG pattern can alert clinicians to this type of seizures/etiology, and facilitates the prompt initiation of precision treatment with sodium channel blockers, even prior to the return of genetic test results, which can typically take up to several weeks. Additionally, in centers where access to broad based genetic testing is not readily or rapidly available, recognition of this aEEG pattern may permit targeted gene testing or at least a clinical diagnosis of channelopathy to help guide clinical management. Finally, the high specificity of this EEG pattern may help increase or decrease suspicion for pathogenicity if there are variants of uncertain significance identified in these genes.

Both on aEEG and cEEG, the attenuation at onset of the seizures and the profound postictal suppression are unique characteristics compared to findings in seizures in neonates due to other more common etiologies [25–27]. No patients from the two comparison groups had a similar pattern on aEEG and cEEG, with exception of those with *KCNQ2*-related epilepsy. Patients in the second comparison group had likely pathogenic variants in the *ATP1A3*, *CACNA1A*, *GABRB3*, *KCNT1*, and *SCN8A*, which are other known genes affecting channel function, and we did not find a similar EEG pattern. However, the comparison group is quite small due to the very low incidence of other channelopathies as causative of seizures in the neonatal period, and we cannot fully exclude that in future more channelopathies will be found to be associated with this ictal EEG pattern. The pathophysiological substrate of this ictal pattern remains unknown and it is hitherto not described in other channelopathies. Remarkably, this EEG feature, also described as an icicle on quantitative analysis of cEEG, is also recently described in a 16-year-old

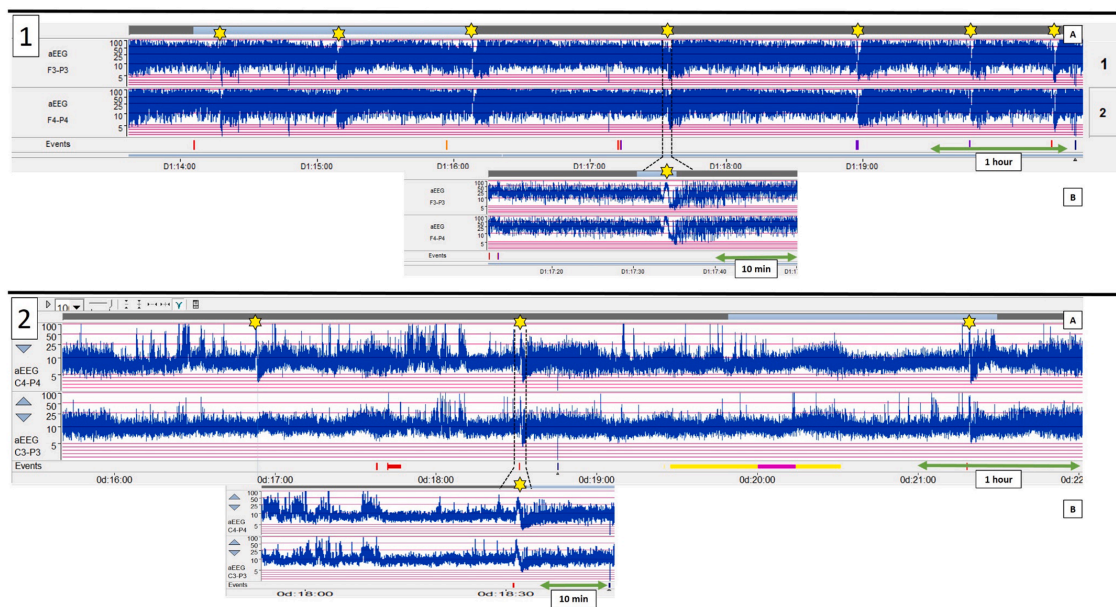


Fig. 1. Characteristic ictal aEEG pattern in *SCN2A* (A) and *KCNQ3* (B)-associated epilepsy.

1 aEEG at a regular paper speed (A. upper panel 6 cm/h) and increased paper speed (B. lower panel 15 cm/h) of patient 1. Seizures are marked by asterisks, showing bilateral synchronous discharge with a sudden decreased, then rise in both the lower and upper margin, followed by the characteristic marked postictal attenuation. 2 aEEG at a regular paper speed (A. upper panel 6 cm/h) and increased paper speed (B. lower panel 15 cm/h) of patient 13. The characteristic pattern, with the sudden rise and postictal attenuation, preceded by a short decrease in amplitude, can be seen although less clear than in sample A.

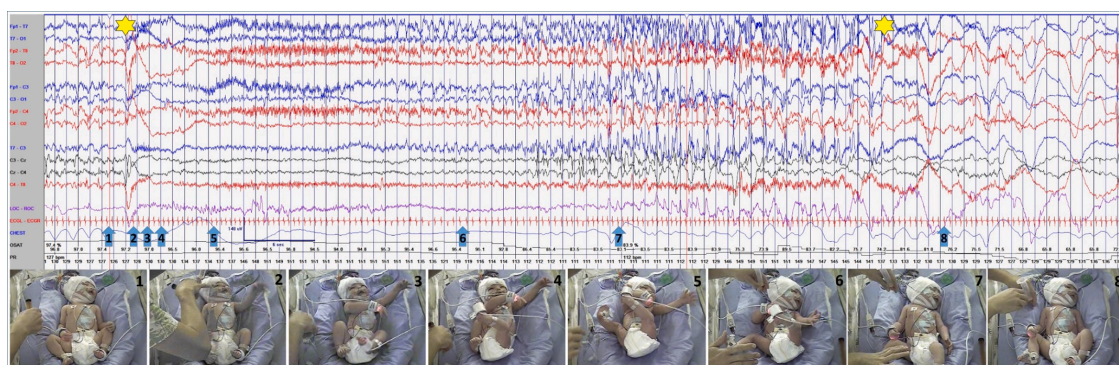


Fig. 2. Ictal EEG recording of a patient with *SCN2A*-associated epilepsy.

Ictal continuous video EEG recording of patient 6 with pathogenic variant in *SCN2A* gene showing the EEG and clinical findings. 1. Pre-ictal phase with normal background activity and resting clinical state. 2 - 5. Seizure onset with quick sequential tonic posturing starting with the left arm (3), right arm (3), left leg (4), and right leg (5) over 6 s with synchronous diffused background attenuation and superimposed low amplitude fast activity. 6 - 7. The patient begins to relax the diffused tonic posturing, decreasing O₂ saturation as product of ictal apnea and the EEG shows gradual increase of cortico-thalamic organization (6) showing rhythmic tonic discharges with a gradual increase of amplitude and frequency of 1 - 3 Hz delta spike and slow wave discharges while the patient became hypotonic (7). 8. Post-ictally, the patient regains muscle tone and spontaneous movements and breathing while the EEG showed diffused attenuation of fast activity with high amplitude very slow waves (<0.25 Hz). EEG settings: LFF 1 Hz, HFF 70 Hz, notch 60 Hz on, sensitivity 7 uV/mm, paper speed 10 mm/sec.

patient with *SCN2A*-related epilepsy [28].

Clinically, seizures in neonates due to genetic variants, particularly channelopathies, can be discerned from seizures provoked by acutely acquired causes by the time of onset and the seizure semiology. The onset of genetic epilepsy is typically after 24 h of life (median 60 h) as opposed to provoked (symptomatic) seizures, which generally start in

the first 24 h of life [29]. Seizure semiology typically comprises focal or sequential-generalised tonic posturing (Fig. 2) in channelopathies [7,8, 29], which was also seen in our case series.

In case of seizures in the neonatal period, it is appropriate to perform a brain MRI, in clinical practice usually preceded by cranial ultrasound, to rule out other causes of seizures [30]. In *SCN2A* channelopathies, MRI

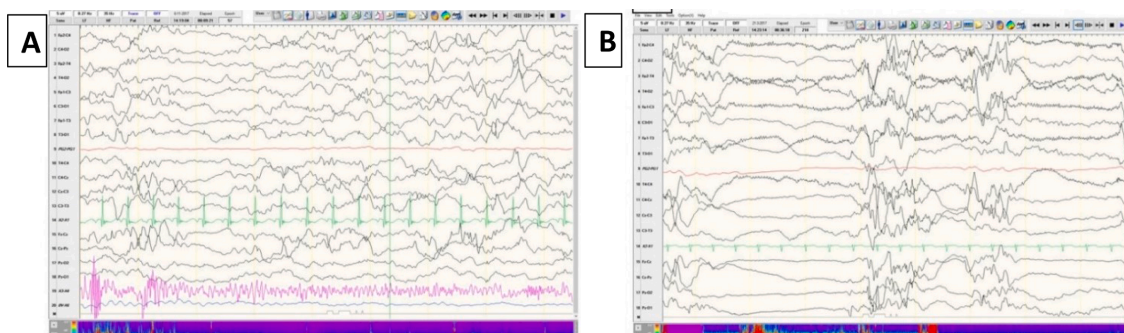


Fig. 3. Interictal EEG patterns in patients with *SCN2A*-related epilepsy.
 A. Almost normal interictal EEG pattern (patient 3) with a continuous EEG activity and brief epochs of background attenuation (trace alternant) in quiet sleep.
 B. Severely abnormal interictal EEG pattern (Ohtahara Syndrome type), with a suppression-burst pattern (patient 1). Bursts consist of mixture of spikes, sharp waves or sharp and slow waves – voltage (100 – 350uV). Frequent inter-burst intervals of 2–4 s length. The duration of suppression was increased during the sleep.
 EEG settings: LFF 0.27 Hz, HFF 35 Hz, notch on, sensitivity 5 uV/mm, paper speed 10 mm/sec.

abnormalities including hyperintensities in brain stem, basal ganglia and white matter abnormalities [31], and a thin or atrophic corpus callosum [19], have been previously described. In our cohort patient 2 had diffusion restriction in the thalamus and caudate, which could be an additional cause for seizures [32]. However, the seizures were not consistently focal, neither responded to conventional ASM, and further suspicion of a genetic cause was raised based on the aEEG pattern, highlighting the utility of recognizing this specific pattern.

The characteristic aEEG/EEG pattern associated with *KCNQ2*, *KCNQ3* and *SCN2A*-related neonatal epilepsies were especially valuable for patients 3–5, 7–8 and 12–13, as bedside recognition of the pattern led to early and effective treatment with a sodium-channel blocker. It was also particularly valuable for patient 10 in this cohort. This patient has a heterozygous *KCNQ3* p.(Arg520*) variant, which predicts a truncating stop gain. However, autosomal dominant *KCNQ3* variants associated with neonatal-onset epilepsies are typically missense substitutions. Although these missense substitutions also cause loss of function, this is not necessarily equivalent to truncation or haploinsufficiency of the channel. Pathogenic truncating variants have been described in the context of autosomal recessive *KCNQ3*-related epilepsy, yet in these reports, heterozygous carrier parents have been unaffected [33,34]. For patient 10, this variant was inherited from the father who had a history of paroxysmal episodes in infancy but with reportedly normal EEG. Therefore, it is unclear whether: 1) the heterozygous p.(Arg520*) variant is causative in a dominant manner with variable penetrance, or 2) there is a second undetected variant on the other *KCNQ3* allele and this individual actually has recessive *KCNQ3* disease, or 3) the patient is only a heterozygous carrier and there is actually another cause for her seizures unrelated to *KCNQ3*. Although the EEG pattern for recessive *KCNQ3*-related epilepsy has not yet been described, the presence of the characteristic aEEG/cEEG pattern confirms that this patient likely does have *KCNQ3*-related neonatal-onset epilepsy, and that a dominant effect from this variant may indeed be possible. This demonstrates that this aEEG pattern and the reaction to sodium channel blockers may be helpful to assist in variant interpretation and to guide management when genetic results are unclear.

To date, mutations reported in the *SCN2A* gene, encoding the $Na_v1.2$ channel, cause different overlapping phenotypes. The clinical phenotype may depend on the type of variant and whether the variant is responsible for loss or gain of function of the $Na_v1.2$ channel or more complex mixed effects [8,35]. Missense mutations with a gain-of-function are typically correlated to an early onset epilepsy (before 3 months), in which the severity of the gain-of-function effect can be correlated to the phenotype severity. Truncations and canonical

splice site mutations, and to a lesser extent missense mutations with a presumable loss-of function effects, typically correlate to later onset of epilepsy (after 3 months) [8,36]. Within this group of patients with late onset epilepsy, some were reported to have a pattern of SWAS (spike-wave activation in sleep) [8]. Interestingly, our patient 1 developed SWAS at the age of 4. As SWAS has been associated with other channelopathies [37], a low threshold of suspicion is warranted and an EEG during sleep should be performed in patients with a known channelopathy and any symptoms suggestive of SWAS (i.e. behavior changes, language regression). Missense mutations in sodium channel *SCN1A* and *SCN2A* also predispose children to encephalopathy with febrile seizures [38]. In our study, patient 3, carrying a de novo likely pathogenic *SCN2A* variant, also had febrile seizures.

In line with previous observations [8,23,36], 9 out of 13 patients in this study became seizure free after initiation of a sodium channel blocker, and 3 patients had a significant seizure reduction. One patient became seizure free with phenobarbitone. In neonates with gain-of-function *SCN2A* mutations, sodium channel blockers often lead to a clinically relevant seizure reduction or seizure freedom, whereas other ASM are less effective [8,36]. The effect is probably caused by inhibition of the increased $Na_{v1.2}$ activity in cells with an excitatory function. [5] In contrast, sodium channel blockers are rarely effective in loss-of-function *SCN2A* variants associated with late onset epilepsy (≥ 3 months), and can even worsen seizures [8,36]. Potassium channels encoded by the *KCNQ2* and *KCNQ3* genes (Kv7.2/3 channels) regulate resting potential and prevent repetitive firing. Pathogenic mutations lead to a loss of this inhibitory effect [39]. The Kv7.2/3 channels and sodium channels are suggested to have a structural and functional co-localization at the neuronal membrane in various regions of the brain [40,41], which might explain the effect of sodium channel blockers in *KCNQ2* and *KCNQ3* associated epilepsy [10,23].

Early seizure control in neonates and children is important, as a higher seizure burden is negatively associated with (long-term) neurodevelopmental outcome [11,12]. A recent meta-analysis did not find a difference in functional neurodevelopment or epilepsy at age 24 months among children whose ASM was discontinued vs continued at hospital discharge after resolution of acute provoked (symptomatic) neonatal seizures [42], but no formal studies have investigated the most appropriate duration of ASM treatment in channelopathies causing neonatal epilepsy. In our study, ASM was discontinued in seven patients (after a maintenance treatment ranging from 6 months to 2.5 years), upon which five children remained seizure free. Seizures recurred in patient 2 after 2.5 years, and patient 1 developed SWAS after 1.5 years (at the age of 4), with a good response to oral corticosteroids. In patient 3, only

provoked seizures occurred after vaccination or viral illnesses.

In conclusion, seizures in neonates with *SCN2A* and *KCNQ3* related epilepsy can be recognized by the same characteristic aEEG ictal pattern as reported in *KCNQ2*-related epilepsy, extending these unique features to other channelopathies. Recognition of this pattern together with seizure semiology facilitates the prompt initiation of precision treatment with sodium channel blockers, even before genetic test results are available. This characteristic (a)EEG pattern may also be valuable in the context of interpreting variants of uncertain significance in *SCN2A*, *KCNQ2* and *KCNQ3* genes.

Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

Declaration of Competing Interest

C. M. P. C. D. Peeters-Scholte is founder and consultant at Neurophyxia BV. She holds several patents and stocks of Neurophyxia BV. None of this work has a relationship with the current manuscript.

The other authors report no conflicts of interest, according to ICMJE recommendations.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.seizure.2023.06.016.

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Article 3

Distinctive Amplitude-Integrated EEG Ictal Pattern and Targeted Therapy with Carbamazepine in *KCNQ2* and *KCNQ3* Neonatal Epilepsy: A Case Series.

- **Ana Vilan**, Ana Grangeia, José Mendes Ribeiro, Maria Roberta Cilio, Linda S de Vries. Distinctive Amplitude-Integrated EEG Ictal Pattern and Targeted Therapy with Carbamazepine in *KCNQ2* and *KCNQ3* Neonatal Epilepsy: A Case Series. *Neuropediatrics*. 2023;55(01):32-41. doi.org/ 10.1055/a-2190-9521. Epub 2023 Oct 12.

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 amplitude-integrated electroencephalography (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 five neonates with KCNQ2/3 pathogenic gene variants admitted at a level 3 neonatal intensive care unit (NICU) over an 8-year period.

Results The distinctive ictal aEEG pattern was recognized in four neonates after an average of 61.5 hours (minimum 12 hours, maximum 120 hours) from the first electro-clinical 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 first and second antiseizure medication, respectively. Three out of five patients with continuous normal voltage (CNV), sleep–wake cycling (SWC), and shorter postictal suppression had normal neurodevelopmental outcome. Regarding the remaining two infants, one was not trialed with CBZ and had a high seizure burden, both presented with a prolonged postictal 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.

Conclusion 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 postictal suppression were associated with normal developmental outcomes.

Keywords

- KCNQ2
- KCNQ3
- neonatal seizures
- amplitude-integrated EEG
- carbamazepine
- postictal suppression

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Introduction

Diagnosing and managing neonatal seizures can be challenging. While most seizures occur in response to a brain insult, in 13 to 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 more than 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 days 3 to 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 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 nonspecific, 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 electroencephalography (EEGs) are considered the gold standard for seizure diagnosis, amplitude-integrated EEG (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 unit (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 15 cm/h, as opposed to the routine 6 cm/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

As 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/continuous EEG (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 five 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 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-EEG machine (NicoletOne, Natus, Seattle, Washington, United States) 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 10–20 system for electrode placement modified for neonates¹⁸ 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 margins 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 (A.V., J.M.R.) for the purpose of the study.

The aEEG tracing was divided into six 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, burst suppression (BS), and flat trace.¹⁰ The frequency of electroclinical seizures was classified as single seizure, repetitive seizures (three or more

Table 1 Clinical, genetic and neurophysiologic features of the 5 patients described in this report

	Patient 1 (index case)/2014	Patient 2/2015	Patient 3/2019	Patient 4/2022	Patient 5/2022
Family history	No	No	No	No	Yes/elder 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 d	10 d	6 d	10 d	6 d
Szs					
Age at Sz onset	36 h	36 h	48 h	20 h	30 h
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, reticollis, facial flushing, salivation followed by unilateral clonic jerk	Bradycardia, bilateral tonic posturing of upper and lower extremities, desaturation followed by high pitched cry	Tonic asymmetric, facial flushing, salivation, desaturation
Sz 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
Unilateral ictal pattern present	Yes	Yes	Yes	No	Yes
Duration (s) of postictal suppression of first recorded Sz	174 s	110 s	96 s	192 s	52 s
Duration (s) of postictal suppression prior to CBZ (mean $\pm$ SD)	n/a	117 (72–112) 89 $\pm$ 17	102.2 (81–118) 100 $\pm$ 13	141.3 (77–192) 141 $\pm$ 35	55.5 (50–63) 55 $\pm$ 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

Table 1 (Continued)

	Patient 1 (index case)/2014	Patient 2/2015	Patient 3/2019	Patient 4/2022	Patient 5/2022
Time of CBZ administration after recognition of ictal aEEG	n/a	96 h	18 h	120 h	12 h
Age at Sz control	30 d	5.5 d	6 d	7 d	2 d
CBZ dosage	n/a	15 mg/kg/d; 8/8 h	20 mg/kg/d; 8/8 h	20 mg/kg/d; 8/8 h	20 mg/kg/d; 8/8 h
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	<i>de novo</i> KCNQ2: c.830C > T, p.(Thr277Ile)	<i>de novo</i> KCNQ2: c.1657C > T, p.(Arg553Trp)	<i>de novo</i> KCNQ3: c.956A > G, p.(Tyr319Cys)	<i>de novo</i> KCNQ2: c.1742G > A, p.(Arg581Gln)	Inherited KCNQ2: c.152del, p.(Gly51Alafs*82)
Outcome					
Neurological examination at discharge	Lack of visual fixation Hypotonia	Lack of visual fixation Axial hypotonia	Normal	Lack of visual fixation Axial hypotonia	Normal
Sz outcome	Recurrence at 1 and 6 y during febrile illness	Recurrence at 3 y upon discontinuation of CBZ	Sz free No recurrence	Recurrence at 29 d, during SARS-CoV-2 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 y	Normal/7 y	Normal/4 y	Global psychomotor delay. Developmental plateau at the age of 3 mo/1 y	Normal/8 mo

Abbreviations: aEEG, amplitude-integrated electroencephalography; ASM, antiseizure medication; CBZ, carbamazepine; CNV, continuous normal voltage; DNV, discontinuous normal voltage; FU, follow-up; GA, gestational age; LEV, levetiracetam; MDZ, midazolam; MRI, magnetic resonance imaging; n/a, not available; NGS, next-generation sequencing; PB, phenobarbital; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; Sz, seizure; TPM, topiramate; VPA, valproic acid.

seizures during a 30-minute period but not SE), and SE (continuously ongoing seizure activity for >30 minutes or recurrent seizures for $\geq 50\%$ of the recording time).¹⁹ The postictal 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 BS or continuous slowing, with suppression below 10 μ V. The postictal amplitude suppression of the first electroclinical seizure was measured. The mean duration of the postictal 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 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 nonresponders (seizures persisted during hospitalization). ASM's effect on electrocortical background activity was observed.

Genetic Investigations

Genetic testing comprised *KCNQ2* next-generation sequencing (NGS) (patients 1 and 2), epilepsy multigene NGS panel (patients 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) and *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 neuro-radiological features are summarized in ►Table 1.

Clinical Features

All infants were born at term, with clinical manifestations within the first 48 hours after birth (median 30 hours, range 20–48 hours). 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 to 9 per day. All patients had seizures characterized by an initial tonic phase with autonomic features. In four patients, the tonic phase was followed by unilateral clonic jerks. Infections and inborn errors of metabolism workup were performed in all infants except patient 5 and were negative.

All infants had an MRI in the first week of life, except patient 5 who only had cranial ultrasound 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 (10 mg/kg intravenous [IV]) at 36 hours of life and then an additional 10 mg/kg IV. A trial of pyridoxine 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 IV 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 30 hours of IV administration (loading dose of 2 mg/kg in 10 minutes, 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 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. She had single seizure, as patient 5 and CBZ was the second and the first 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. She was transferred at 72 hours of life to our unit with one single electroclinical seizure in the first 24 hours 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 margins followed by amplitude suppression. Interictally four patients had a CNV background and one a DNV. At 15 cm/h, the ictal pattern and the postictal suppression were more easily recognized (►Fig. 1). Postictal amplitude suppression of the first electroclinical seizure ranged between 52 and 192 seconds. The longest duration of suppression (192 seconds) was observed in the infant with a DNV pattern. SWC was present in three patients. Patients with CNV had bilateral and unilateral ictal patterns (►Fig. 2).

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. 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

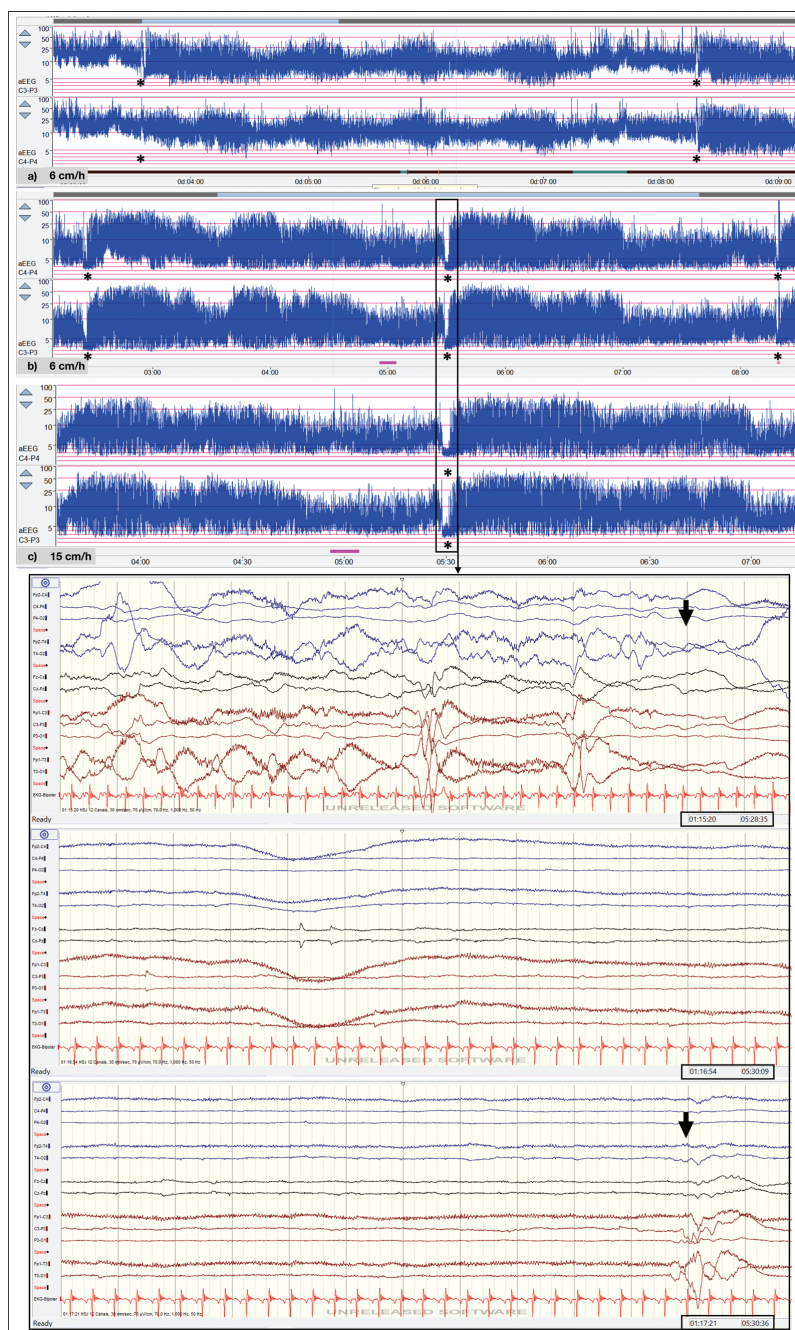


Fig. 1 Distinctive aEEG ictal pattern (asterisks) on a CNV background pattern with SWC (A), and on a DNV background pattern without SWC (B), at 6 cm/h. At 15 cm/h, the ictal pattern and the post-ictal suppression is more easily recognized (C). On the cEEG, the duration of the post-ictal suppression is depicted. The beginning and the end (arrows), corresponds in the lower right corner to 05:28:35 and to 05:30:36 in time, respectively, corresponding to 121 seconds of duration.

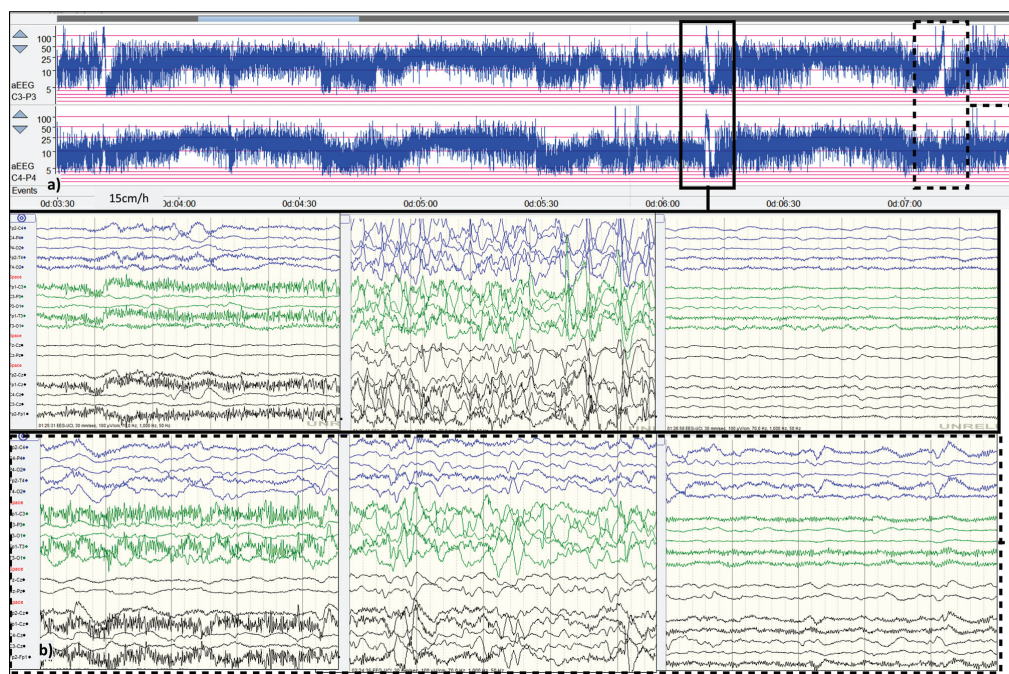


Fig. 2 Distinctive aEEG bilateral and unilateral ictal patterns (dotted line) on a CNV without SWC at 15 cm/h (A). Evolution of the corresponding cEEG during the seizure confirms bilateral vs unilateral ictal patterns (B).

presented with repetitive episodes of desaturation and clonic jerks in the first 48 hours 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 autistic features. At 5 years 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, patients 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 two seizure recurrences at 1 and 6 years after a febrile illness. He is seizure free till now on VPA and levetiracetam. At 9 years of age, he has intellectual disability, no language and autistic features.

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

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

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 five 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.

KCNQ2/3-related epilepsy has an onset in the first day 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 recognized. 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 recommends two-channel aEEG/cEEG or multichannel cEEG with dual channel aEEG (aEEG/cEEG) monitoring to confirm clinical seizures and monitor efficacy of treatment. Continuous 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 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 four patients and targeted therapy with CBZ was brought forward, ranging from 12 to 120 hours after the first 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 margins rather than a predominant prolonged postictal 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 postictal 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 15 cm/h and with simultaneous analysis of the raw EEG and measurement of the postictal suppression. The observation of a diffuse EEG flattening or attenuation following a postictal 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 postictal suppression and neurodevelopmental outcome is reported. We believe that the length of postictal suppression could be used as a clinical 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 the first-line ASM irrespective of seizure etiology, except for a family history that suggests channelopathy, then a SCB should be the first-line ASM.²⁵ All seizures were timely detected in our case series, and PB was the first-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. SCB including CBZ, OXC, 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 is the most effective ASM in SLNE, and considering side effects, CBZ is suggested as first choice when suspicion is high. In DEE, PB was ineffective as first-line ASM. SCB, VPA, and levetiracetam were partially effective without significant differences.²⁶ In patient 1, CBZ was not trialed but the last ASM to introduce was VPA. In patients 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 first ASM in one patient and the second 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 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 month 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 patients 1 and 4 a DEE phenotype.

Patient 1 had a higher seizure burden with multiple ASM and was not trialed to CBZ. The impact of seizure burden on cognition and behavioral 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 1 year of age, 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. 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 her family history (asymptomatic father; sister with neurological disability and autistic 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. Patients 1 and 4 had the longest postictal 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 postictal amplitude suppression.

Conclusion

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.

The next step is to have access to early biomarkers that could help 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 postictal 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.

Conflict of Interest
None declared.

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4. DISCUSSION

DISCUSSION

Brain monitoring in the NICUs is mandatory. Recognizing all the difficulties involved in the diagnosis of seizures in neonates, electrophysiological monitoring, either conventional or aEEG, has become an important tool in the NICUs. Although aEEG has become a standard monitoring tool in the management of APS in the neonatal period, little is known about the usefulness of aEEG in the context of neonatal epilepsy. Our research focused on the role of aEEG in the diagnosis and management of channelopathies, including *KCNQ2/3* and *SCN2A*-related neonatal epilepsy.

1. aEEG and *KCNQ2*-related epilepsy

Our journey started in 2014 with our first patient – index case, the first child of healthy unrelated parents, without any familial history of epilepsy. At 36 hours of age, he presented with tonic seizures characterized by asymmetric tonic posturing with head deviation, eye blinking, facial flushing followed by unilateral clonic jerks and desaturation. He was treated with phenobarbital and seizures resolved till day 7 when he had a cluster of similar seizures and was transferred to our unit. Continuous brain monitoring with aEEG/cEEG was started soon after admission and he went through an exhaustive number of investigations (infection work-up, metabolic panel, MRI and MR spectroscopy) and polytherapy (phenobarbital, pyridoxine, levetiracetam, midazolam, lidocaine, clonazepam, topiramate, valproic acid) as he evolved to status epilepticus. No etiology was found at that moment, and the most intriguing aspect was the electroclinical phenotype of the infant with a peculiar aEEG ictal pattern. A genetic testing was only ordered at three months of age that revealed a *de novo* *KCNQ2* pathogenic variant. He developed a profound intellectual disability with autistic features. One year later, a female infant, the first child of healthy nonrelated parents, without family history of neonatal seizures was admitted to our unit because of repetitive tonic asymmetric posturing with head deviation unilateral clonic jerks and salivation. aEEG monitoring was initiated shortly after admission, revealing the same aEEG ictal pattern. Under aEEG monitoring, a trial of carbamazepine was administered at four days of life, resulting in seizure control without recurrence. A *KCNQ2* single-gene sequencing was ordered and revealed a *de novo* pathogenic variant in *KCNQ2*. She had a normal developmental outcome.

In the first work of this thesis, we conducted a multicenter retrospective study to evaluate the ictal aEEG patterns in neonates with *KCNQ2*-related epilepsy. We performed a detailed analysis of the clinical, neuroradiological and genetic characteristics of 9 newborns that were admitted because of neonatal seizures. Continuous aEEG was used in all infants and careful analysis of the ictal discharges revealed a distinct aEEG seizure pattern, consisting of a sudden rise of the lower and upper margins of the aEEG of short duration immediately followed by a marked suppression

of the aEEG amplitude in 8 of our 9 patients. The simultaneous analysis of continuous cEEG allowed us to exclude possible artifacts and also the full characterization of the ictal electroencephalographic aspects on the aEEG reflecting the pattern on the cEEG, including descriptions of the onset, evolution and termination with a profound and prolonged postictal suppression (Figure 3).

The EEG morphology of ictal termination include simple reversion to a normal background,

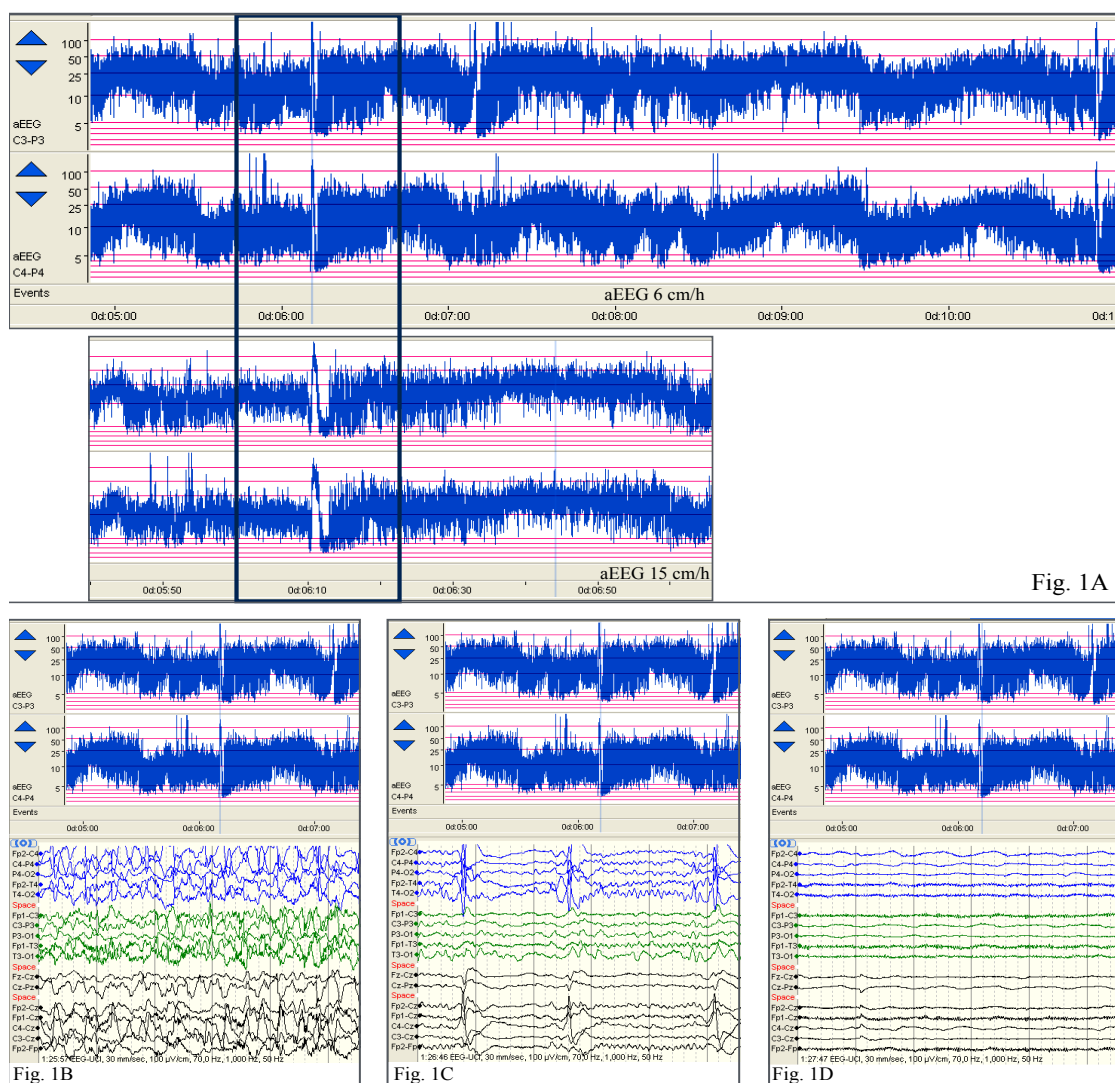


Figure 3. Findings by Vilan et al. (article 1)

generalized or focal postictal slowing, generalized or unilateral EEG suppression and periodic discharges^(102, 103). In our report, all the seizures terminated with a profound and prolonged postictal suppression. Postictal generalized EEG suppression (PGES) has been defined as a generalized, severe attenuation of EEG (amplitude $<10 \mu\text{V}$), during $\geq 10 \text{ s}$, apart from muscle, movements, respiratory and electrode artifacts, starting in the immediate aftermath or within 30 s after the ictal pattern termination⁽¹⁰³⁾. This pattern of markedly reduced cortical activity following an ictal event is rarely reported in children⁽¹⁰⁴⁾. In 2006, Kim et al, defined it as an abruptly attenuated terminal (AAT) ictal pattern, and reported that it was

more common in children with idiopathic rather than symptomatic epilepsies, and not present before 6 months of age⁽¹⁰⁵⁾. The first report of a postictal marked diffuse voltage attenuation related with genetic epilepsy, was published in 2014, in a small case series of 3 newborns with de novo missense mutations in the *KCNQ2* gene, studied with prolonged video-EEG monitoring⁽¹⁸⁾. Later, this finding was confirmed in a relatively large cohort of newborns with genetic epilepsy, 12 with pathogenic variants in *KCNQ2*, 1 *KCNQ3* and 2 *SCN2A*⁽¹⁰⁶⁾.

Although this was a retrospective work with limited number of patients, this is to our knowledge, the first study to describe a distinctive ictal aEEG pattern in neonates with *KCNQ2*-related epilepsy. The ictal discharges were brief and were more clearly depicted with less time compressed recordings at 15cm/h, followed by the postictal amplitude suppression.

Also, we provided an electroclinical phenotype consisting of sequential seizures comprising asymmetric tonic posturing, often followed by unilateral or bilateral clonic movements associated with desaturations and the reported ictal aEEG patterns and demonstrated under aEEG monitoring, that in the two most recent cases, prompt recognition of this electroclinical phenotype at the bedside led to targeted treatment with carbamazepine with termination of seizures while awaiting genetic testing confirmation.

In all patients, various ASM (range 2-8) were trialed before adequate seizure control was achieved, denoting the drug-resistant epilepsy. Phenobarbital was the first-line ASM in all patients being effectiveness in one patient. Midazolam, lidocaine, pyridoxine and levetiracetam were used as second-line. The sodium channel blocker lidocaine was trialed in six patients and deemed partially effective. In the two most recent cases, oral carbamazepine was the fourth and fifth line ASM, when the neonatologist recognised the distinct ictal aEEG pattern. The clinical observation about the efficacy of carbamazepine in patients with *KCNQ2*-related epilepsy has already been reported ^(18, 49, 50) but this is the first report demonstrating the carbamazepine efficacy under aEEG monitoring with seizure cessation and with no recurrence.

More recently, our findings were confirmed by other authors⁽¹⁰⁷⁻¹⁰⁹⁾. In the case report by Kwak et al in 2020, a female newborn with *KCNQ2* encephalopathy showed the same distinctive ictal aEEG pattern while monitoring in the NICU and not targeted to carbamazepine. The authors reinforce the importance of early recognition of this electroclinical phenotype to guide not only the genetic testing but also to targeted treatment with carbamazepine while waiting for the genetic results⁽¹⁰⁹⁾.

Dilena et al reported three members of a family (father and offspring) with neonatal onset *KCNQ2*-related epilepsy and how useful the recognition had been of the distinctive ictal aEEG pattern in the more recent case with use of carbamazepine as first line ASM with rapid seizure control and short hospitalization⁽¹⁰⁷⁾.

Kazazian et al. reported two newborns with complex congenital heart disease and *KCNQ2*-related epilepsy and they compared the two cases concerning electroencephalographic brain monitoring. The first case was monitored only with cEEG, with poor control of seizures until genetic diagnosis of *KCNQ2* gene variation was confirmed at 3 months of age and ASM was

switched to carbamazepine. The second case was immediately monitored with aEEG and this not only aided in confirming that the paroxysmal events were indeed seizures but also facilitated the identification of the aEEG pattern as being consistent with *KCNQ2*-related epilepsy. As a result, carbamazepine was promptly started, leading to the cessation of seizures. The authors highlight the importance of bedside aEEG monitoring with recognition of the distinctive ictal aEEG pattern and prompt treatment with carbamazepine⁽¹⁰⁸⁾.

We consider that our initial observation later substantiated by other reports⁽¹⁰⁷⁻¹⁰⁹⁾ greatly contributed to the early recognition of the neonatal phenotype of *KCNQ2*-related epilepsies guiding the diagnostic workup including genetic testing and early treatment with carbamazepine while waiting for the genetic results.

2. aEEG and *KCNQ3* and *SCN2A* – related epilepsy

KCNQ2, *KCNQ3* and *SCN2A* genes have been associated with a spectrum of phenotypes, ranging from SLNE to EIDEE, mostly presenting with sequential seizures, characterized by asymmetric tonic posturing associated with apnea and desaturation⁽¹⁹⁾. Also, these channelopathies characteristically present a postictal amplitude suppression in EEG recordings⁽¹⁰⁶⁾. We hypothesized that the same ictal aEEG pattern observed in *KCNQ2* would be present only in *KCNQ3* and *SCN2A*, and not in other genetic epilepsies or APS.

We carried out an international multicentre observational case series of 13 neonates (9 neonates with *SCN2A* variants and 4 neonates with *KCNQ3* variants) and using two comparison groups (APS and genetic epilepsies group) we could observe that the ictal aEEG pattern that we previously have described in *KCNQ2*-related epilepsy was only present in all except one neonate with *SCN2A*- and *KCNQ3*-related epilepsy.

Tonic sequential seizures are seen in channelopathies⁽¹⁹⁾. Cornet et al.⁽¹⁰⁶⁾ highlight the importance of seizure semiology in clarifying the etiology of seizures in neonates. In their study, they observed that tonic sequential seizures were presented in channelopathies, namely *KCNQ2*, *KCNQ3*, *SCN2A*, *KCNT1* and *PRRT2*, but when cEEG recordings were analyzed, the postictal suppression was only presented in *KCNQ2*, *KCNQ3* and *SCN2A*.

In our study, we performed a careful analysis of the ictal aEEG pattern using a comparison group of neonates with pathogenic variants affecting ion channel function (*KCNQ2*, *ATP1A3*, *CACNA1A*, *GABRB3*, *KCNT1* and *SCN8A*) and, apart from neonates with *KCNQ2*-related epilepsy none of the other channelopathies presented the distinctive ictal aEEG pattern characterized by the brief rise in lower and upper margins followed by a pronounced postictal suppression that we believe that it is a hallmark of *KCNQ2/3* and *SCN2A*.

A previous study of Lee IC et al.⁽¹¹⁰⁾, compared ictal aEEG pattern of 5 newborns with *KCNQ2* variants with ictal aEEG pattern of 11 newborns with non-*KCNQ2* seizures (n=9 APS, n=1 neurofibromatosis type 1, n=2 non-*KCNQ2*) and concluded that the ictal aEEG pattern is different in the two groups and is helpful for early diagnosis and treatment of *KCNQ2*-related epilepsy. Surprisingly, the authors do not highlight the postictal suppression.

With a larger sample, we compared 82 neonates with seizures due to HIE and treated with therapeutic hypothermia (electroclinical seizures $n=55$, and/or electrical seizures without clinical correlate $n=75$) with 13 neonates with seizures due to pathogenic *SCN2A* and *KCNQ3*-variants, and conclude that the ictal aEEG pattern is different. In APS there is a gradual rise of the upper and lower margin not followed by the postictal suppression. Also, the transient rise in APS lasts longer as opposed to the brief rise in *KCNQ3* and *SCN2A* that is best observed in less time compressed (from 6 to 15cm/h), as we have documented in *KCNQ2*-related epilepsy.

In our study, no patients from the two comparison groups had a similar pattern on aEEG and cEEG, with exceptions of those with *KCNQ2*-related epilepsy, confirming that this ictal aEEG pattern is a unique and characteristic of these channelopathies.

Once more, various ASM were trialed before adequate seizure control. In 7 out of 13 neonates, a SCB was the second or third line ASM used after bedside recognition of this distinct aEEG ictal pattern.

3. Clinical practice of aEEG in diagnosis of *KCNQ2/3*-related epilepsy

Gathering all the evidence of the role of the aEEG in the diagnosis of channelopathies and early target therapy with carbamazepine, we decided to evaluate whether neonatologists were aware of this ictal pattern concerning time to recognition after the first electroclinical seizure and start carbamazepine.

We conducted an observational single-center case series of *KCNQ2/3*-related epilepsy, and for the purpose of ictal aEEG pattern recognition we calculated the time interval from the first electroclinical seizure and time to recognition for the attending neonatologist and targeted treatment with carbamazepine. aEEG was monitored continuously after admission and we observed that we took an average of 61.5 hours (minimum 12 hours, maximum 120 hours) to recognize the distinctive aEEG pattern and target therapy with carbamazepine. Several characteristics of our sample could explain this wide range, namely:

i) Age at seizure onset.

In our cohort, the neonates had clinical seizures between 20 hours to 48 hours of life. Age at seizure onset is usually used as an indicator for differentiation between APS and epilepsy syndromes with onset in neonates. Seizures due to HIE or stroke usually begin in the first one or two days of life as opposed to unprovoked seizures that typically start between days 2 and 7 of life⁽⁵⁶⁾. The age at seizure onset may have contributed to the fact that genetic epilepsy was not considered earlier in the differential diagnosis. Although this is a small cohort, we think that age at seizure onset should be used carefully when considered in the differential.

ii) Seizure frequency.

In *KCNQ2*-related epilepsy, characteristically seizures are brief but very frequent, occurring up to 30 times/day or even evolving to status epilepticus in SLNE and up to 35 seizures per hour in EIDEE^(19, 20). In our case series, seizure frequency was low. It is not clear why, probably this could be explained by the use of ASM. All except one had a loading dose of phenobarbital

(10-20 mg/kg) prior to the initiation of aEEG. Phenobarbital is the most commonly used and studied first-line ASM in more than 90% of neonates with seizures, regardless of etiology, with a variable response rate^(6, 7).

The effect of phenobarbital in *KCNQ2*-related epilepsy has been evaluated in two systematic reviews that concluded that it might be considered in self-limited epilepsy^(111, 112). In our cohort there was no response to phenobarbital, but this ASM could contribute to the low seizure frequency in our case series that is not in agreement with the literature^(19, 20).

Although this is a small sample, we think that it is interesting to note that we didn't observe any electroclinical dissociation, a phenomenon that is well known in infants with APS, like HIE, usually after phenobarbital administration⁽¹⁴⁾.

iii) Seizure duration

The duration of the seizure is one of the characteristics that can influence aEEG recognition⁽⁸²⁾. In the compressed aEEG trend, brief seizures can be difficult to detect but with devices where the speed can be adjusted, this may improve aEEG ictal pattern recognition. Using less time compressed, 15cm/h, the transient rise in the aEEG amplitude can be well depicted. Further research is necessary to assess whether using less time compressed in brief seizures, will improve aEEG sensitivity for seizure detection.

iv) inter-rater reliability for the recognition of aEEG ictal pattern

The current data regarding the accuracy and reliability of aEEG for neonatal seizure detection is variable^(82, 113, 114). Although the same methodology was used for seizure detection, which was recognition of a transient rise in aEEG amplitude, maximum and minimum border and the confirmation with the raw trace, the ability for detection of seizures on aEEG depends on the level of experience of the neonatologist. Several studies comparing inter-rater agreement for seizure detection on aEEG concluded that having readers with at least three years of experience increase the levels of reliability⁽¹¹³⁻¹¹⁵⁾. Although aEEG was conceived to be a simple tool to be applied and interpreted by the NICU staff, adequate training is an essential part of monitoring⁽¹⁵⁾. We didn't compared inter-rater agreement for seizure detection on aEEG, but we think that we have to improve the training in our unit. At the moment we have a minimal training of 4 hours per year to medical staff and nurses.

Another particularity that might contribute to explain this wide range to the delay in recognition, 12-120 hours, is the prolonged postictal suppression that is also part of the ictal aEEG pattern and we think that most of the neonatologists are not aware of it. Future studies, combining seizures in the newborn and aEEG, may help to introduce this distinctive ictal aEEG pattern. The characteristics of this ictal aEEG pattern can be challenging for less experienced observers. Our study is an opportunity to training in reading this ictal aEEG pattern.

As soon as the ictal aEEG pattern was recognized targeted treatment with carbamazepine was started under aEEG monitoring, at daily doses ranging from 15 to 20 mg/kg/day with prompt response. One strength of our work was the use of continuous aEEG monitoring not only for the diagnosis of seizures but also for assessment of ASM efficacy. Remarkably, all patients, except

our index case were seizure free by the end of the first week of life, since they were targeted to carbamazepine as soon as the distinctive ictal aEEG pattern was recognized.

4. aEEG beyond ictal pattern in *KCNQ2/3*-related epilepsy

The aEEG was devised to be a simple tool, easy to apply and with the ability to monitor continuously for long-term providing information on brain activity^(68, 69). Besides seizure detection, aEEG gives an overview of trends of brain function and maturity and easily identifies the sinusoidal patterns characteristic of SWC⁽⁷⁰⁾.

In critically ill neonates, not only early assessment of the aEEG background patterns but also ongoing continuous recording informing the trajectory of recovery over time has prognostic value ^(92-100, 116).

We evaluate the aEEG background activity and the presence or absence of SWC in our cohort. We observed that 3 infants with continuous background activity and SWC had normal neurodevelopmental outcome.

In our last work, we verified that 2 infants with the worst neurodevelopmental outcome had absent SWC, one of them with high seizure burden and continuous background activity and the other with a discontinuous background activity.

In term infants, a continuous aEEG pattern with cyclic variations or with a fully developed SWC, is usually a reassuring sign of brain integrity⁽⁸⁹⁾.

The SWC is a biological concept describing the regular pattern alternating between periods of sleep and wakefulness⁽¹¹⁷⁾ that it is well depicted in the aEEG where a discontinuous part of the characteristic sinusoidal pattern represents quiet sleep while the more continuous aEEG periods represent wakefulness/active sleep⁽⁸⁹⁾.

Polysomnographic monitoring remains the gold standard for determining infant sleep states; however, it requires long periods of time and is often difficult to perform in NICU populations⁽¹¹⁸⁾. In a recent study comparing aEEG with polysomnography, the authors concluded that aEEG may be suitable to monitor sleep states⁽¹¹⁹⁾.

Sleep is a highly sophisticated brain function and is known to be important in the process of neural networks and synaptic plasticity for memory consolidation and emotional processing⁽¹²⁰⁾. The newborn ability to spend time in sleep, or alternate between sleep states is important to lifelong neurological well-being^(93, 120) and several studies have shown that the infant's ability to express SWC are strongly predictive of later neurodevelopment^(96, 121, 122).

We demonstrated that using aEEG, SWC can be easily recognized and 2 infants with absent SWC had impaired neurodevelopment.

Our study opens a new possible application for aEEG in channelopathies with evaluation of SWC as possible biomarker of cerebral dysfunction.

We also observed that a discontinuous aEEG background pattern was present in a term infant, with Apgar score of 9 and 10 at 1st and 5th minute, respectively, with no sedative medications

during recording besides 2 doses of phenobarbital (10 mg/kg+10 mg/kg at 20 hours of life). She started aEEG recording at 72 hours of life with discontinuous background pattern with no recovery over time. She also had absence of SWC.

In term infant periods of mild discontinuity can be seen during quiet sleep⁽¹²³⁾ or due to sedative medications^(124, 125). It can also be a non-specific finding due to brain immaturity, mild encephalopathy or metabolic abnormalities⁽¹²⁶⁾. Increased time with a discontinuous background activity is predictive of adverse outcome for newborns with neurological dysfunction⁽¹²¹⁾. We believe that in this particular case, discontinuous aEEG background pattern is an abnormal pattern and may be suggestive of brain dysfunction.

There is no data available regarding the accuracy of aEEG in interpretation of background pattern and SWC in *KCNQ2/3*- related epilepsy.

Several reports using electroencephalographic characteristics in the assessment of outcome in *KCNQ2* have been reported^(20, 127, 128). Casas-Alba and collaborators recently demonstrated the correlation between epileptic activity and developmental outcome in a cohort of patients with *KCNQ2*-related epilepsy⁽¹²⁷⁾. EEG characteristics, for example, burst-suppression pattern or multifocal epileptiform activity are more frequently related to EIDEE. Two reports mentioned that sleep cycles on cEEG were indistinguishable in patients with moderate or severely impaired development^(128, 129). Future research using aEEG monitoring in channelopathies with regard to assessment of the background pattern and the presence of SWC is of utmost relevance.

Finally, we took a detailed analysis of postictal aEEG amplitude suppression, concerning the duration prior to start of carbamazepine and we observed that the longest duration of postictal aEEG amplitude suppression was presented in infants with worst neurodevelopmental outcome. The duration ranged between 52 seconds to 192 seconds.

Postictal EEG suppression is the most characteristic postictal state and it has been found in 84% of seizures in 94% of adult epilepsy patients⁽¹³⁰⁾. The underlying mechanism of postictal EEG suppression remains unknown. It may be related to anoxia, acute hypercapnia, or cortical spreading depression^(103, 131, 132). In children and adults with chronic uncontrolled epilepsy, postictal generalized EEG suppression (PGES) is a possible identifiable EEG marker of profound cerebral dysfunction and when severe can lead to central apnea and death^(103, 133-137). Several factors that could facilitate the occurrence of PGES in this population have been analyzed, including state of wakefulness, age of seizure onset, duration of the seizure and ASM tapering⁽¹³²⁾. The presence and duration of a tonic phase is an independent predictor of PGES⁽¹³¹⁾ and peri ictal autonomic dysfunction, particularly hypoxemia has been observed⁽¹³¹⁾. Lhatoo et al. reported in a case- controlled study of adult patients with refractory epilepsy undergoing resective surgery or vagal nerve stimulator implantation, that PGES was significantly longer in the generalized motor seizures, with prolonged PGES (> 50 seconds) significantly associated with a higher risk of sudden unexpected death in epilepsy (SUDEP) and quadrupled when the duration was greater than 80 seconds⁽¹⁰³⁾.

Subsequent studies add more evidence that PGES could be a biomarker of SUDEP⁽¹³⁴⁻¹³⁶⁾, although some ambiguity remains⁽¹³³⁾.

It was of interest to observe that the presence and duration of PGES may be an electroclinical marker of cerebral dysfunction.

There is no data about postictal aEEG pattern. What we have found in our work is a distinctive aEEG ictal pattern characterized by a sudden rise of the lower and upper margins of the aEEG followed by a marked suppression of the aEEG amplitude in all ictal discharges. There are some similarities with the previous literature, namely the tonic phase and the autonomic dysfunction (apnea and desaturation). No infant in our study so far subsequently died of SUDEP, although the duration of postictal amplitude suppression ranged between 52 seconds to 192 seconds. Further clinical studies are recommended to clarify the meaning of this aEEG postictal amplitude suppression in *KCNQ2/3* and *SCN2A*, that we think that is also part of the ictal aEEG pattern and it might be related with the channel dysfunction. Also, it would be interest to analyze which cut off could be used to predict worst neurodevelopmental outcome.

This is the first time that a possible correlation between the duration of the postictal amplitude suppression and neurodevelopmental outcome in *KCNQ2/3*-related epilepsy is reported.

Although limited by number of cases, we think that this study is unique. We intended to provide biomarkers as early as possible – the neonatal period, that can help to predict the long-term outcome.

5. aEEG and genetic testing in *KCNQ2/3* and *SCN2A*-related epilepsy

After clinical recognition and further electroencephalographic confirmation of seizures, an etiological investigation should be initiated. A complete prenatal, birth and postnatal history is followed by a careful examination in an attempt to clarify if there were provoking factors or a genetic predisposition. Genetic etiology may be suggested by the presence of a family history of neonatal epilepsy^(19, 36).

What we have found through our research was that in a total of 24 infants, only 4 had a positive family history and we identified 8 de novo pathogenic variants in *KCNQ2* gene, 1 de novo pathogenic variant in *KCNQ3* gene and 7 de novo pathogenic variants in *SCN2A*. De novo monoallelic mutations in *KCNQ2/3* and *SCN2A* are an increasingly recognised cause of early-onset seizure and developmental delay^(20, 22, 138).

Our work highlights the importance of recognizing this electroclinical phenotype and considering genetic testing early in the investigation, avoiding an extensive workup, even if the family history is negative. As genetic testing becomes more prominent in the etiological investigation, the chances of reaching an etiological diagnosis in neonatal onset epilepsies are expected to be rising⁽¹³⁹⁾.

Although it is increasingly acknowledged that unprovoked seizures may be genetically based, access to genetic testing remains limited. Availability of testing relies on economic and scientific resources and differs across regions globally and test results may not be available within a short period of time to allow tailored treatment⁽³⁶⁾. All genetic results in our research, except one that was based on prenatal screening, were available beyond the neonatal period. *KCNQ2/3*

and *SCN2A*- related epilepsies have implications for tailored treatment, as carbamazepine and others SCB have proven effective in controlling seizures^(18, 48–51, 53). Our work highlights the importance of recognizing this electroclinical phenotype and start carbamazepine while waiting for the genetic results.

Recent progress in genomic technologies (comparative genome hybridization array, targeted next-generation sequencing and WES), has revealed hundreds of previously unidentified pathogenic variants in individuals with neurodevelopmental disorders, including epilepsy⁽¹³⁹⁾.

It is of interest to note that in the beginning of our journey we started with single-gene testing and then with the increasing availability of next generation sequencing technologies and more recently WES, we have moved away from targeted single-gene analysis. This widespread genetic testing was crucial for our research, as electroclinical prediction of causative genes is challenging due to genetic heterogeneity that we observed in the *KCNQ2*, *KCNQ3* and *SCN2A* genes.

A major concern is that using a large targeted panel may lead to the identifications of VUS, whose interpretation is challenging due to limited data on pathogenicity in genetic databases and the absence of functional studies⁽³⁶⁾. Careful phenotyping can be useful in interpreting the genetic finding⁽¹⁴⁰⁻¹⁴³⁾ as was the case with one infant with a novel missense *KCNQ2* variant (C.1065C>Gp.Asp355Glu) that was classified as pathogenic according to its position in the gene and the patient's phenotype.

We moved a little further and we could verify that the high specificity of this aEEG pattern may help increase suspicion for pathogenicity if there are VUS identified, as was the case in an infant with a heterozygous *KCNQ3* p. (Arg520*) variant. This unique electroclinical phenotype may help clinicians in interpreting phenotype-genotype correlation and classify VUS as clinically diagnostic.

5. CONCLUSIONS

CONCLUSIONS

The following conclusions from this thesis can be drawn:

1. aEEG is a complementary tool to cEEG in the diagnosis and management of genetic epilepsies.
2. We observed that in *KCNQ2*, *KCNQ3* and *SCN2A* – related epilepsy presenting with sequential seizures, a distinct ictal aEEG pattern can be recognized.
3. We described the distinct ictal aEEG pattern that is characterized by a brief rise of the lower and upper margins of the aEEG followed by a postictal amplitude suppression and demonstrated that when using less time compressed (15 cm/h) this is more easily recognized.
4. Clinical observations have proven the efficacy of carbamazepine, a SCB, in seizure control of patients with *KCNQ2*-related epilepsy. We demonstrate that recognition of this electroclinical phenotype – seizure semiology and ictal aEEG pattern, at the bedside, prompt early and effective targeted treatment with carbamazepine while awaiting genetic testing.
5. We demonstrated that this ictal aEEG pattern is unique to these channelopathies and is not present in other genetic epilepsies or in APS.
6. This electroclinical phenotype may help clinicians consider early genetic testing during the investigation, even if the family history is negative.
7. Due to the genetic heterogeneity of these channelopathies Sanger-sequencing is not the best approach for genetic testing and WES should be performed instead.
8. This distinct ictal aEEG pattern may also be valuable in the context of interpreting variants of uncertain significance in *KCNQ2*, *KCNQ3* and *SCN2A* genes.
9. We analyzed the aEEG background pattern and the presence or absence of SWC. We observed that discontinuous aEEG background pattern and absence of SWC were present in newborns with worst neurodevelopmental outcome.
10. We further analyzed the duration of postictal aEEG suppression. We demonstrated that the longest duration was presented in infants with the worst neurodevelopmental outcomes.

6. FUTURE DIRECTIONS OF RESEARCH

FUTURE DIRECTIONS OF RESEARCH

We suggest the following in improving our understanding of aEEG in diagnosis and management of *KCNQ2/3* and *SCN2A*-related neonatal epilepsies:

1. Increase the awareness of this ictal aEEG pattern

The aEEG is becoming a clinical standard monitor tool in many NICUs worldwide and progress has been made in seizure detection with the emergence of digital systems and the possibility of simultaneous analysis of the raw-EEG from which the aEEG trend is created. Although, the utility of aEEG in seizure detection is very dependent not only on the risk of seizures but also on the level of experience when interpreting aEEG. Dissemination of this ictal aEEG pattern through conferences and teaching sessions of aEEG training might increase the awareness of this channelopathy seizure pattern.

We have analyzed our model of teaching in our unit and concluded that it was insufficient. Integrated into a research project starting in September 2025 – a quality improvement intervention to increase seizure detection in real time using aEEG in the NICU – one of the defined interventions is a 6-hour training module with periodic reassessments.

2. Studying whether early seizure control with carbamazepine under aEEG monitoring, has a positive effect on long-term neurodevelopmental outcome

Understanding the effects of seizures on brain function and their potential to cause lasting deleterious changes in the developing brain remains a hot topic. We have demonstrated that it is possible to have seizure control by the end of the first week of life. Larger studies using the same methodology are necessary to analyse if this time window of intervention will affect long-term neurodevelopmental outcome.

3. Studying aEEG biomarkers concerning neurodevelopmental outcome: length of postictal suppression; aEEG background pattern and presence of SWC

Larger cohorts of infants with *KCNQ2/3* and *SCN2A* – related epilepsy are needed to evaluate whether aEEG background patterns, the presence or absence of SWC and length of postictal suppression predict further outcome.

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