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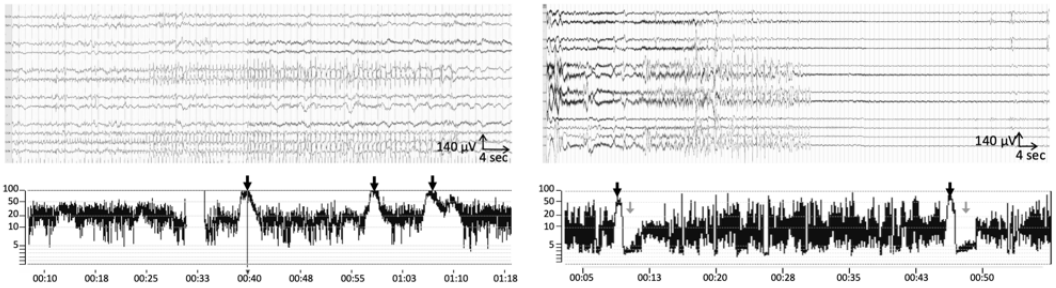

A novel approach to seizures in neonates:

From acute provoked seizures to ultra-rare epilepsies

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ABBREVIATIONS

ACNS: American Clinical Neurophysiology Society

AD: Autosomal Dominant

ADC: Apparent Diffusion Coefficients

APS: Acute Provoked Seizures

AR: Autosomal Recessive

ASM: Antiseizure Medicine

BFNE: Benign Familial Neonatal Epilepsy

BS: Burst Suppression

CSF: Cerebrospinal Fluid

DEE: Developmental Epileptic Encephalopathy

DNA: Deoxyribonucleic Acid

DOL: Days of life

DWI: Diffusion-Weighted Imaging

aEEG: Amplitude-integrated EEG

cEEG: Conventional EEG

vEEG: Video EEG

EEG: Electroencephalogram

EIMFS: Epilepsy of Infancy with Migrating Focal Seizure

EME: Early Myoclonic Encephalopathy

FCD: Focal Cortical Dysplasia

HC: Head Circumference

HIE: Hypoxic-Ischemic Encephalopathy

ID: Intellectual Disability

MOL: Months of life

MRI: Magnetic Resonance Imaging

MRS: Magnetic Resonance Spectroscopy

NE: Neonatal Encephalopathy

NICN: Neuro-Intensive Care Nursery

NICU: Neonatal Intensive Care Unit

OS: Ohtahara Syndrome

SE: Status Epilepticus

S(F)NE: Self-limited (Familial) Neonatal Epilepsy

SSRI: Selective Serotonin Reuptake Inhibitors

TH: Therapeutic Hypothermia

UCL: Universite Catholique de Louvain

UCSF: University of California San Francisco

WES: Whole Exome Sequencing

WGS: Whole Genome Sequencing

CHAPTER 1: FROM NEONATAL SEIZURES TO SEIZURES IN NEONATES: CONTRIBUTORS TO THIS RAPIDLY EVOLVING FIELD

INTRODUCTION

Seizures are common in the neonatal period affecting 1 to 3 per 1000 births, and are responsible for a wide array of neurodevelopmental impairment [1,2]. Seizures during this epoch, defined as the period from birth to 28 days of life in term infants, or 44 weeks post-conceptional age in preterm infants, are due to a wide range of etiologies [3]. About 80% of seizures in neonates result from an acute event and are called acute provoked seizures [4]. The most common cause of provoked seizures in neonates is hypoxic-ischemic encephalopathy (HIE). In HIE, reduced flow of oxygenated blood to the brain before or during delivery leads to brain dysfunction [5]. Other causes of provoked seizures are stroke, infection, or acute electrolyte and glucose imbalances [4]. However, it has been recently recognized that in ten to twenty percent of neonates, seizures are the presenting symptom of a neonatal-onset epilepsy [6].

The recommendations of the American Clinical Neurophysiology Society in 2011 to monitor all neonates at high risk for seizures with long-term video-EEG [7], as well as the development of brain-oriented neonatal intensive care units (NICU), have led to enhanced collaboration between neonatologists and neurologists resulting, for the first time, in the delineation of specific electro-clinical phenotypes based on etiology [8–10]. This should allow us to move from the first neonatal-onset epilepsies to be recognized more than thirty years ago, *i.e.* Benign Familial Neonatal Epilepsy (BFNE), Ohtahara syndrome (OS), and Early Myoclonic Encephalopathy (EME), to more discrete etiology-specific diseases [11,12].

In this work, we examined how the etiology of seizures affects the subsequent seizure risk, management, prognosis, and outcome in neonates. We aimed to move the needle from the diagnostic of “neonatal seizure” as a single entity to an etiology-specific approach that considers seizures in neonates as a symptom of different diseases requiring a personalized approach. We started by analyzing frequent causes of neonatal seizures, focusing on seizures in the setting of hypoxic-ischemic encephalopathy. We then reviewed the literature on the genetic causes of neonatal seizures. We identified and described a group of neonates with genetic epilepsies and refined the early electro-clinical presentation of these infants. We identified four neonates with an ultra-rare developmental and epileptic encephalopathy associated with *BRAT1* gene mutations. *BRAT1* gene was not yet included in the targeted epilepsy gene panels at the time, hence the mutation was identified by non-selective NGS approach that whole-exome sequencing represents. Our concept that seizures need to be carefully described and that etiology should be assessed at the same time as treatment is started or even before is novel as it allows for targeted management of these infants.

PART A: THE DEVELOPMENT OF NEURO-INTENSIVE NEONATAL CARE

During the last two decades, with the advent of therapeutic hypothermia (TH) to treat hypoxic-ischemic encephalopathy (HIE), a new area of neuromonitoring and neuroprotection has emerged.

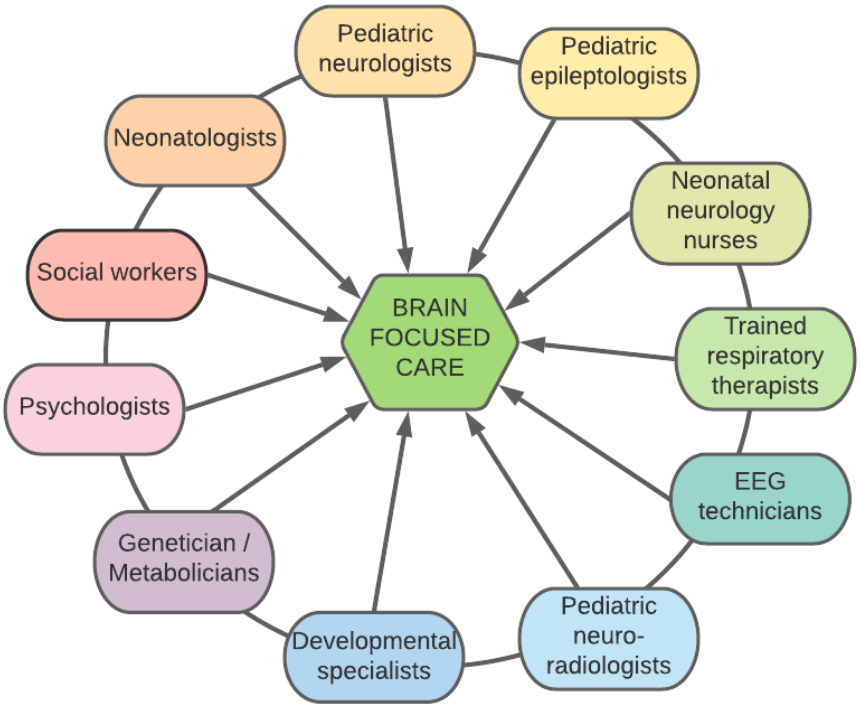
Implementing therapeutic hypothermia has required neonatologists to switch from an individual practice to a multidisciplinary 'brain-focused' approach. The neuro-intensive care nursery (NICN) is a model of co-management of at-risk newborns by a multidisciplinary team including neurologists, neonatologists, and specialized nurses, among others. This model was first developed at the University of California San Francisco (UCSF) and has gained considerable traction, particularly in North America, with many units reporting their experience of setting up similar models of care [13–15]. Central to the treatment of newborns with or at risk of brain injury is the availability of neuromonitoring around the clock, including conventional or video electroencephalogram (cEEG or vEEG) monitoring, amplitude-integrated EEG (aEEG) monitoring, and state-of-the-art MRI, providing a basis for rapid diagnosis and personalized treatment. Given the progress in understanding the evolution of brain injury, the impact of neonatal seizures, and the benefit of appropriate interventions, the NICN becomes indispensable in adopting new standards of care. It is also a platform for research and further dissemination of knowledge.

Neuro-intensive care is an established subspecialty that combines expertise in neurology and intensive care medicine. Adults and children with severe neurological or neurosurgical conditions receive specialized neurological monitoring and clinical expertise, with an emphasis on 'brain-focused care' and outcomes rather than survival only. In neuro-intensive care units, all providers are continually aware of the potential neurological complications of critical illnesses and how their management may be influencing the brain [14,16]. This culture shift is achieved through the education of all providers and the development of specialized medical and nursing teams that work together using standardized management guidelines.

The first Neuro-intensive care nursery (NICN) opened its door in July 2008 at the University of California at San Francisco (UCSF) Benioff Children's Hospitals. This was made possible by strong institutional support and commitment from stakeholders in neonatology, child neurology, neonatal nursing, neurophysiology/ epileptology, neuroradiology, genetics, developmental, and follow-up care (Figure 1). The neuro-intensive care nursery offers the possibility of rapid implementation of therapeutic hypothermia and other neuroprotective strategies around the clock by an experienced team. It provides detection and treatment of seizures

guided by standardized protocols, continuous brain monitoring, head ultrasound with high-performance sondes, and high-quality brain MRI. Parental counseling by experienced physicians and nurses and referral for intervention services and follow-up for careful developmental monitoring to mitigate the long-term effects of early brain injury are also essential in the NICN.

Figure 1: Structure of the Neuro-Intensive Care Nursery (NICN) team at UCSF[1]



In neonates, the multidisciplinary team must pay particular attention to the impact of the critical illnesses and management strategies on the developing brain. The goal of neuro-intensive care is to minimize the effect of the initial insult or underlying condition through supportive and interventional clinical management to prevent ongoing or secondary brain injury, implement neuroprotective strategies, and minimize adverse neurological outcomes after a primary insult,

[1] Unless mentioned otherwise, figures and graphics are the creation of the author

such as traumatic brain injury, ischemic stroke, intracranial hemorrhage, and global hypoxia [15]. This neurocritical care approach involves routine management of physiology, combined when appropriate with intensive neurophysiology monitoring of brain activity, monitoring of cardiovascular parameters and laboratory values, as well as detailed brain imaging using advanced techniques such as MRI. This innovative approach allows for the recognition of neurological conditions, prevention of secondary brain injury, rapid identification, and treatment of neurological complications, such as seizures, leading to a better understanding of diagnosis, extent of injury, and prognosis in neonates.

Another aspect of the care of neonates is the need for careful consideration of gestational age and appropriate developmental care. Indeed, the rapid development of preterm and term infants means that interpretation of results and care should be made taking into consideration the infant's maturity.

PART B: THE ADVENT OF MODERN ELECTROPHYSIOLOGIC MONITORING IN NEONATES

Despite the evolution of new technologies for assessing neonatal brain function, electroencephalography (EEG) remains one of the most valuable diagnostic and prognostic tools. EEG is the gold standard for distinguishing epileptic seizures from nonepileptic paroxysmal events and detecting subclinical seizure activity in high-risk neonates [17,18].

In the past decade, the field of EEG monitoring has made tremendous progress due to the increased possibilities of data elaboration and display brought by digitalization. The use of continuous video-EEG has now entered everyday clinical practice in many NICUs, leading to improved brain function assessment, more accurate neonatal seizure diagnosis, rapid treatment, and improved prognosis[19].

In 2011, the American Clinical Neurophysiology Society published updated guidelines recommending to monitor all neonates at high risk for seizures with continuous video-EEG for at least 24 hours and 24 hours after the last seizure [7]. The authors of these updated guidelines recognized that there might be significant practical barriers to implementation, particularly regarding equipment availability and technical and interpretive personnel. The recommendations were framed as “an expression of idealized goals and not a mandated standard of care.”

The progressive application of these idealized goals in neonatal units around North America and Europe has allowed the recognition that most seizures in neonates are subclinical [20,21], that aEEG is neither sensitive nor specific enough to detect seizures accurately [22–24], and that use of continuous video EEG monitoring decreases seizure duration [15,21], MRI brain injury and exposure to ASM[21]. Therefore, continuous EEG with simultaneous video recording is now considered the standard of care in most institutions [19] for prolonged continuous monitoring over multiple hours or days. It is regarded as the gold standard for seizure diagnosis, seizure characterization, and quantification of seizure burden in newborns. It allows clinicians to determine the exact localization of seizure onset and their propagation and provide detailed information on brain maturation, acute or remote brain injury, prognosis, and response to ASMs [25].

Conventional EEG and particularly continuous conventional EEG is resource-intensive and is not available in every institution on a 24-hour basis. There is also limited availability of expertise in performing and interpreting EEG in the neonatal period. On the other hand, with the development of new therapeutic strategies for the treatment of hypoxic-ischemic encephalopathy (HIE), such as hypothermia, there has been significant interest in generating alternatives strategies for the

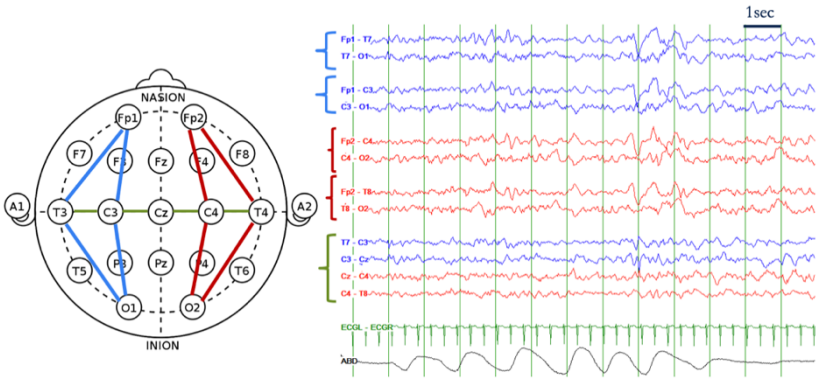
continuous assessment of brain function in the neonate, including trend analysis of EEG recordings such as amplitude-integrated EEG (aEEG). While accurate for background assessment and outcome prognostication, seizure detection with aEEG is limited, and aEEG should be used as a screening tool for seizure only as it is less sensitive and specific than the "raw" EEG [26]. The combined use of both EEG and aEEG provides the benefits of displaying aEEG on the bedside monitor to assist neonatologists in prompt decision-making and allow revision and interpretation of the full montage by neonatal neurologists or neurophysiologists for subsequent diagnostic confirmation or useful refinements.

1. TECHNICAL ASPECTS

Neonatal EEG recordings should cause the least possible distress to the newborn to reduce interference with behavior. To properly assess the neonatal EEG, it is necessary to interpret it considering the gestational age at the time of the recording, the behavioral state, the clinical data including medication history, and the presence and depth of therapeutic hypothermia.[27]

The American Clinical Neurophysiology Society (ACNS) recommends the application of 9 scalp electrodes according to the International 10 to 20 System modified for neonates (Fp1, C3, T3, O1; Fp2, C4, T4, O2; Cz - Figure 2) [18].

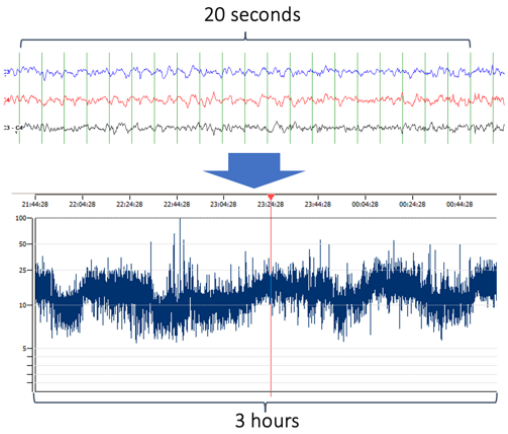
Figure 2: Polygraphic EEG montage in neonate and example of a typical term tracing



aEEG is a simplified method of continuous cerebral function monitoring that has become a standard clinical practice in the NICU as neonatologists and bedside nurses can easily interpret it. aEEG is an EEG-based bedside brain-monitoring tool [26,28,29]. It displays one or two channels of EEG data after filtering, rectification, and smoothing on a semilogarithmic scale (Figure 3).

Figure 3: aEEG vs. EEG in the NICU

- Filtered and compressed 1-2 channel raw EEG
- Allow pattern recognition by the bedside team
- Evaluation of long-term changes and trends in electro-cortical background activity
- Evaluation of seizure activity



2. GOALS AND INDICATIONS OF EEG MONITORING

Continuous video EEG is a better predictor of outcome than neurologic examination in neonates who are severely ill. On EEG, the background patterns, even more than the presence or absence of seizures, correlate significantly with long-term outcomes[30]. The prognostic value of neonatal EEG has long been recognized in both term and preterm infants. Its value can be increased by obtaining early recording (e.g., within the first 48 hours of life and before administration of antiepileptic drugs), by prolonged recording to include samples of different activity states, and, when continuous monitoring is not available, by prolonged serial EEGs at short intervals to assess rapid changes that are likely to occur in high-risk infants. Different drugs such as sedatives and opiates, frequently used in the neonatal intensive care unit (NICU) can alter the EEG background [31,32]. Those effects must be considered in the interpretation of neonatal EEG.

EEG should be considered in any neonates with an unexplained altered neurological examination (encephalopathy), when there is a suspicion of seizure, or in the setting of specific risk factors (e.g., HIE, central nervous system infection, brain malformation, intracranial hemorrhage, cardiac surgery). Many neonates, particularly the most severely affected, have no clinical correlate to their seizures (electrographic seizures), hence vEEG monitoring is essential for diagnosis and treatment of seizures [33]. The distinction between epileptic and non-epileptic movement can be difficult, especially in neonates with movements such as jerks, pedaling, or lip-smacking. These movements need to be recorded and confirmed to be seizures to avoid unnecessary use of ASM.

According to the ACNS [18], monitoring of high-risk infants should be performed for a period of at least 24 hours. EEG is mandatory to monitor the effectiveness of antiseizure medicine, given the high rate of electro-clinical dissociation. Indeed, in up to 58% of neonates with persistent seizures after ASM, the clinical component is absent, and seizures are subclinical only [34]. Once the proper response, which is the absence of EEG seizures, has been achieved, it is recommended to continue EEG monitoring until the patient has been seizure-free for 24 hours; it is also advisable to monitor for seizure recurrence during and after discontinuation of ASMs. EEG helps assess the functional extent of brain injury by evaluating the degree of abnormality of background EEG patterns and, especially with serial recordings, predict neurologic outcomes according to its dynamic evolution over time. This has been extensively studied in acute HIE but holds for different etiologies such as infection or acute brain injury.

PART C: THE PROGRESS OF GENETIC TESTING

Up to 80 % of early-onset epilepsies are due to pathogenic genetic variants [6,35,36]. The early identification of pathogenic genetic variants in patients with epilepsy is essential to provide an accurate prognosis and to optimize management. This includes the use of precision medicine as targeted therapeutics emerge [37]. As genetic testing is becoming more widely available, our ability to identify genetic causes of epilepsy is improving. There is a wide array of genetic testing available, and the strategy should be made based on the suspected underlying pathophysiologic mechanism. Of note, even in cases of epilepsy acquired in the setting of an acute brain injury (e.g. stroke, infection), genetic background likely influences whether or not epilepsy develops [38].

1. RISKS AND BENEFITS OF GENETIC TESTING AND DIAGNOSIS

The International League Against Epilepsy (ILAE) Genetics Commission recommends weighing the risks and benefits of genetic testing according to the ACCE (analytic validity, clinical validity, clinical utility, and ethical, legal, and social implications) framework [39]. Analytic validity refers to the accuracy of the test and has to do with the inherent limitations of the test itself. Clinical validity refers to the predictive value of the test in the situation under consideration, which has to do with the population selected, limitations of predicting phenotype from genotype, and the likelihood that a particular phenotype will manifest. Clinical utility includes ways in which a diagnosis would affect management. It includes how a genetic diagnosis may affect treatment decisions but also whether it might reveal potential comorbidities that require specialized surveillance and management and how it reduces testing associated with an ongoing diagnostic odyssey. Ethical, legal, and social implications encompass other ways in which the patient and family may be affected by the results [40].

Of note, reaching a precise genetic diagnosis, even when no medical treatment is available, has multiple implications that impact families and children. A genetic diagnosis enables detailed individual and familial genetic counselling. For example, it allows to better predict the recurrence risks to different family members and enables parents to receive recommendation on indication of reproductive alternative options, if indicated. Indeed, while for diseases with an autosomal recessive mode of inheritance, there is a 25% recurrence risk as for *BRAT1* genetic mutations; when identification of a ‘de novo’ mutation in an autosomal dominant condition is identified, the risk of recurrence is extremely low. Reaching a precise genetic diagnosis also allows parents to join support and advocacy groups. Importantly, a definite genetic diagnosis may alleviate parental anxiety and guilt.

Based on the ability of non-selective NGS approach to identify uncertain results, incidental findings, and false paternity among others, the risks need to be carefully discussed with the family before any genetic testing is performed. Studies have demonstrated very different perceptions about genetic testing and its potential for benefit and harm across families [41].

2. MODALITIES OF GENETIC TESTING

The type of genetic study offered depends on the electro-clinical presentation, the family history, and the other paraclinical information (metabolic testing, head ultrasound, MRI results among other).

Table 1: Types of genetic testing, advantages and disadvantages, reproduced from Sands et al.[40]

Table 4 Genetic tests. An overview of the advantages and limitations across currently available genetic tests. *CNV* copy number variant, *del/dup* deletion or duplication, *WES* whole exome sequencing

Test	Detects	Does not detect	Notes
Cytogenetics			
Karyotyping	Aneuploidy Balanced translocations Ring chromosomes CNV >5–10 Mb	No CNV <5–10 Mb	May be the only way to detect a ring chromosome Required for detecting balanced translocation as the parental source of copy number variation
Microarray	CNV >100–300 kb	No large chromosomal alteration CNV <100–300 kb	For most purposes has supplanted karyotyping; SNP arrays detect absence of heterozygosity
Mixed			
Gene panels	Deep reads (>50–100×) Flanking intronic sequences Gene and exon level del/dup	Only select genes are evaluated	Most sensitive look but only at select genes
Next-generation sequencing			
Whole exome sequencing	Coding sequences Flanking intronic sequences Del/dup <50–100 bases	~90% coverage at read depth >10× Non-coding sequence not covered	Uneven coverage +/−Mitochondrial sequences Secondary findings
Whole genome sequencing	All sequences and CNVs	~90% coverage at ~10×	Not widely clinically available Coding and non-coding sequence Even coverage, but generally lower depth than WES Mitochondrial sequences Secondary findings

2.A. CYTOGENETIC STUDIES

Cytogenetic studies evaluate copy number variants (CNV) that may span many genes. Those CNVs can be deletions or duplications. There are two main types of cytogenetic studies: standard (conventional) karyotype analysis and chromosomal microarrays.

Conventional karyotype analysis provides a full genome analysis by visually inspecting every chromosome (number and structure) under the microscope after specialized staining techniques. It allows the detection of all aneuploidies, structural rearrangement, deletions, and duplications larger than 5 to 10 million base pairs, depending on the banding technique. It is the validated diagnostic technique to detect distinctive chromosomal rearrangements (e.g., ring chromosome 20) and is particularly important for identifying parental balanced translocation. Knowing the source of copy number variation is particularly important for genetic counseling purposes.

Chromosomal microarrays determine CNVs with higher resolutions (up to 60kbs in the postnatal setting). There are two types of chromosomal microarray techniques used for identifying chromosomal imbalance in the clinical setting: comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays.

CGH-based arrays (aCGH) compare the quantity of genomic DNA in a patient's sample with the genomic DNA in a normal control sample. Single nucleotide polymorphism (SNP) arrays use DNA probes that derive from regions in the genome that show differences between individuals at a single base pair site. These sites are referred to as SNPs. By contrast to CGH arrays, SNP arrays compare the patient sample with numerous normal control samples individually run, normalized, and combined to create a reference set [42]. SNP microarrays can reveal stretches of DNA in which heterozygosity is absent, indicating parental consanguinity or uniparental disomy (the inheritance of both copies of segments of DNA from one parent) both situations that can elevate the likelihood of a recessive condition resulting from homozygosity in these regions. The resolution and subsequent diagnostic yield of a specific array is directly linked to the number, size, and types of probes utilized on the array, as well as their distribution across the entire genome. There are distinct nuances in the analysis and reporting criteria across clinical laboratories, all of which are impacted by the choice of the array platform utilized. For special situations with a known family history of a rare CNV, prior contact with the laboratory is critical to establish whether the platform used by the laboratory can detect the specific familial rare CNV [43]. The relevant family history and any previous clinical reports should be provided to the laboratory in advance for corroboration of findings.

2.B. SEQUENCING STUDIES

Next-generation sequencing (NGS) encompasses many techniques that result in high-throughput analysis of DNA sequences. While with Sanger sequencing, single short DNA fragments are sequenced, with NGS massive sequencing of millions of

short DNA fragments happen in parallel. The approach involves first breaking the DNA into fragments and then amplifying and sequencing each fragment. The million of short sequence reads that result are then aligned to a reference genome. The number of reads at a given base position is referred to as the “depth of coverage.”

NGS can be used to analyze the exome consisting of the coding regions of all ∼20,000 genes representing 1–2% of the genome (whole exome sequencing, WES). Because some DNA fragments are more difficult to amplify and sequence, the coverage is typically about 90% of the exome, with some genes having very low coverage, while others are 100% covered [44].

In panels, NGS is used to sequence a limited set of genes associated with the clinical presentation. Gene panels attempt to ensure excellent read depth (i.e., “deep sequencing”), including flanking intronic sequences and may evaluate for intra-genic deletions and duplications through supplementation with Sanger sequencing and hybridization approaches. Single gene tests use Sanger sequencing, and this technique remains the gold standard for confirmation of NGS findings [45].

However, sequencing technologies that give base-level resolution cannot currently substitute for the information about copy numbers provided by cytogenetic techniques. Therefore, if sequencing is pursued first and is unrevealing, a microarray should be considered.

3. RESULTS INTERPRETATION

Variants are differences in nucleotide sequence when comparing a patient’s sequence from extracted DNA to a reference sequence extracted from exome and/or genome reference sequences. Some variants are known to be deleterious by nature and disease-causing while some variants are benign or have, so far, uncertain or unknown significance. WES will detect between 20,000 and 24,000 variants for any given individual, most of which are considered benign. A trio testing consists of testing both parents and the patient. This strategy related to the huge numbers of variants will allow to classify the changes as possibly ‘de novo’ if absent to both parents DNA; ‘compound heterozygous/ homozygous if present in heterozygous state to each parents DNA (confirming autosomal recessive inheritance or X linked if present to maternal DNA variants, and possibly X linked if present/absent to maternal DNA). Based on available diagnostic techniques, pathogenic mutations consist of frameshifts, small insertions or

deletions (“indels”), splice-site mutations, and nonsense or nonsynonymous missense mutations that cause deleterious changes in the gene.

To be reported pathogenic or likely pathogenic, the affected substitution in the gene must already be reported as pathogenic based on nucleotide substitution, protein change, predictive bioinformatic tools on pathogenicity; possibly expression studies and already published in patients with similar phenotype and thus described to cause the patient’s phenotype. The nature of the variation and its functional consequence should match the genetic mechanism of the disease. Databases of known variants, such as ClinVar, are still incomplete and imperfect, but they are being actively curated as information is updated. Pathogenic mutations are generally not expected to be present in large databases of normal controls for example ExAC, Genome Aggregation Database (gnomAD) or Varisome. Inherited pathogenic mutations should segregate affected and unaffected family members. Variants with insufficient evidence of pathogenicity may be reported as “variants of uncertain significance.” Guidelines have been designed to balance the genetic evidence for pathogenicity of variants and help in the classification and interpretation of variants [46].

Negative test results do not imply that the patient does not have a genetic etiology. Depending on the selected population, only a third to 80% of patients with a suspected early-onset epileptic encephalopathy tested will get a genetic diagnosis [6,36,47]. Pre-test counseling is critical for setting expectations, including the limitations of testing as well as the dynamic limitations of our understanding of the results. Negative results may arise from inherent limitations in testing approaches, from human error, and from the limitations of our knowledge. In situations where a specific condition is strongly suspected, it is worth communicating those suspicions to the laboratory performing the testing, ideally before ordering the test but certainly after receiving a negative result, as they may perform additional testing or analysis. The situation is analogous to communicating suspicions to the neuroradiologist so that studies are protocolled appropriately, and attention directed to maximize the utility of the study.

PART D: NEW CLASSIFICATION OF SEIZURES IN NEONATES

Most seizures in neonates are provoked by an acute illness or brain insult with an underlying etiology either documented or suspected. They are hence called acute provoked seizures (APS).

On the other hand, epilepsy is defined as any of the following conditions: 1. at least two unprovoked seizures occurring >24 hours apart; 2. one unprovoked seizure and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures; or 3. diagnosis of an epilepsy syndrome [48,49]. Epilepsy syndromes may present in the neonatal period, and, with the increasing availability of genetic testing, an expanding number of neonatal epilepsies with genetic and metabolic etiologies is recognized [6]. Although many causes can give rise to seizures in neonates, a relatively small number accounts for most seizures. The Neonatal Seizure Registry, an alliance of US centers that apply the ACNS guidelines for EEG monitoring of high-risk newborns, has found that the leading cause of seizures in neonates is HIE followed by acute ischemic stroke and intracranial hemorrhage (Table 2) [4].

Table 2: Etiology of seizure among 611 neonates enrolled in the Neonatal Seizure Registry (NSR) [4]

Etiology	Frequency
Hypoxic-ischemic encephalopathy	38%
Ischemic stroke	17%
Intracranial hemorrhage	13%
Epileptic encephalopathy	6%
Intracranial infection	6%
Brain malformation	4%
Transient metabolic	3%
Benign familial epilepsies	2%
Other/Unknown	11%

1. HISTORY OF NEONATAL SEIZURES CLASSIFICATION

The first effort to classify neonatal seizures was published by Volpe in 1977 [50]. This classification based on the clinical presentation only, included multifocal clonic, focal clonic, tonic, myoclonic seizures as well as subtle seizures. Subtle seizure was defined as autonomic nervous system changes including variation in respiratory rate, vasomotor changes, salivation, heart rate, and blood pressure as seizure manifestations or polymorphic and atypical clinical events such as staring,

sudden awakening and alerting, eye deviation, eye blinking, nystagmus, chewing, and limb movements such as swimming, rowing, and pedaling.

Correlating contemporaneous visual analysis of clinical seizures, EEG and polygraphic measures, Watanabe and colleagues recognized a wide range of motor, behavioral, and autonomic signs and provided detailed electroclinical correlations [51]. Using video-EEG recordings, Mizrahi and Kellaway also documented electroclinical correlations and noted that many clinical events previously reported as seizures were in fact non-epileptic [52]. Events such as generalized tonic episodes and the so-called subtle seizures, both of which occur without EEG correlate, could be provoked by stimulation and suppressed by restraint. This led to a reconsideration of the classification of neonatal seizures based on pathophysiology (epileptic vs. nonepileptic); electroclinical relationships (electroclinical, clinical only, electrical only); or behavioral (focal clonic, focal tonic, myoclonic, spasms, generalized tonic, motor automatisms) each with additional modifiers to suggest whether they were considered of epileptic or nonepileptic origin. The term motor automatisms included ocular movements, oral-buccal-lingual movements, and “progression movements of the limbs” (pedaling, swimming, rowing).

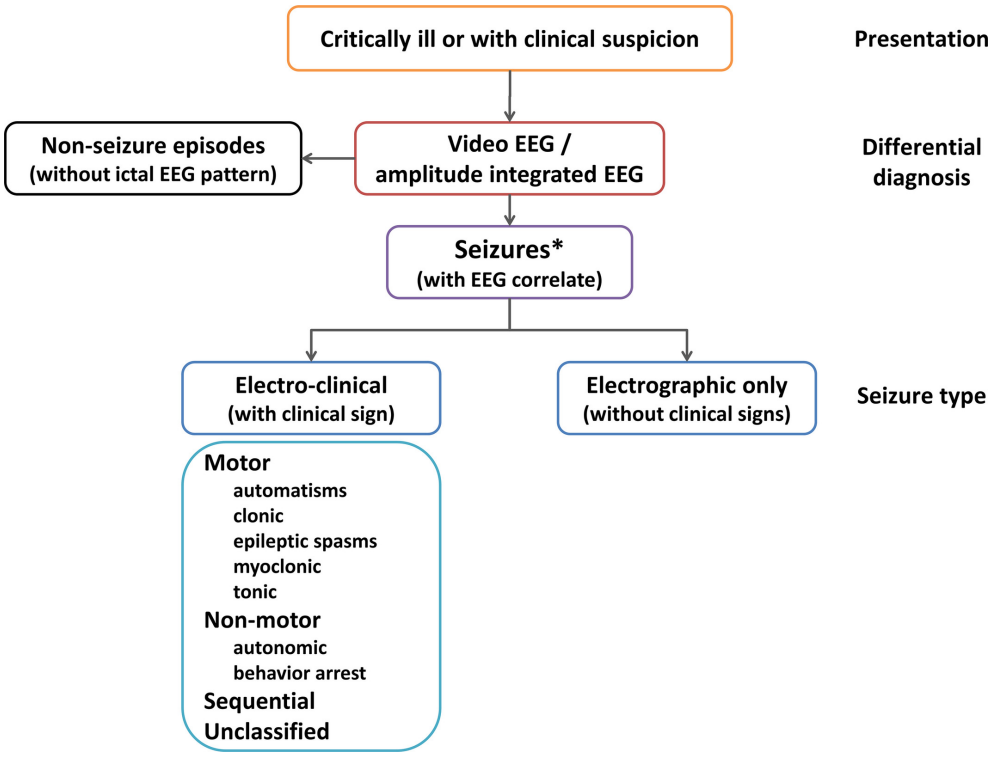
With the advent of prolonged bedside electrographic monitoring in the neonatal intensive care unit (NICU), it has been increasingly recognized that electrographic-only seizures without clinical correlates are frequent, particularly in critically ill neonates. Nevertheless, the 2017 ILAE “Position Papers on Classification of Seizure Types and the Epilepsies” presented a framework for classification including seizure types, epilepsy types, and syndromes that defined seizure as “a transient occurrence of signs and/or symptoms due to abnormal, excessive or synchronous neuronal activity in the brain” [48,53]. Electrographic-only seizures are not included in this definition.

Seizure semiology is the description of signs and symptoms associated with an ictal event and is valuable in localizing the epileptogenic zone. In neonates, the development within the limbic system with its connections to the midbrain and brainstem is more advanced than the cerebral cortical organization, which may, at least in part, account for some differences in neonatal seizure semiology compared to that in older children. In 2014, the ILAE Commission on Classification & Terminology [54] recognized that seizures in the neonate require special considerations, and therefore a Neonatal Task Force was established with the aim of integrating seizures and epilepsies in this age group.

2. 2021 CLASSIFICATION OF SEIZURES AND THE EPILEPSIES IN THE NEONATE

The neonatal task force established a diagnostic framework of seizure in the neonatal period, including a new classification that recognizes that neonates could present both electroclinical and electrographic only seizures (Figure 4) [49]. Electrographic-only seizures are highly prevalent in critically ill neonates (often ventilated, sedated, and treated with muscle relaxants in intensive care). Only events with EEG correlates are included in this classification. Theoretically, focal seizures originating from subcortical cerebral areas such as the limbic and peri-limbic systems may be missed. However, this notion is not, at present, provable or disprovable.

Figure 4 : 2021 Diagnostic framework of neonatal seizures [49]



In contrast with the 2017 ILAE classification [53], because seizures in neonates have been shown to have a focal onset exclusively, the distinction between focal and generalized seizure was deemed not necessary. The difference based on patient awareness was also removed as it is not applicable in neonates.

Seizures are subdivided into motor and non-motor. Although seizures in neonates can present with various clinical signs, in most cases, a single predominant feature can be determined. The task force recommended classifying seizures according to the predominant clinical manifestation. This may or may not be the first clinical manifestation. Because in some situations, it may be challenging to identify the predominant feature (for example, in long seizures where a sequence of clinical features can be seen), sequential seizures were added to the classification. The term sequential seizure can be used in prolonged seizures where a sequence of clinical features is seen.

The task force recommended that the full description of a seizure include the manifestation, a descriptor, and an etiological diagnosis.

Hence, the definition of a seizure in the neonatal period is “An electrographic event with a pattern characterized by sudden, repetitive, evolving stereotyped waveforms with a beginning and end. The duration is not defined but has to be sufficient to demonstrate evolution in frequency and morphology of the discharges and needs to be long enough to allow recognition of onset, evolution, and resolution of an abnormal discharge” [49].

The task force acknowledges that as EEG is not always available, different degrees of seizure probability should be used to reflect the uncertainty of the diagnosis better. Based on the Brighton collaboration, cEEG confirmation is the gold standard for seizure diagnosis [55]. aEEG seizure and focal clonic or focal tonic seizures are considered probable seizures if observed by an experienced professional. All other clinical manifestations need an EEG diagnosis as they represent possible seizures. The task force recommends to avoid treating seizures before EEG confirmation when they are only suspected based on the Brighton collaboration [55].

CHAPTER 2: BRAIN MONITORING TO PREDICT LATE SEIZURE OCCURENCE IN NEONATES WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY

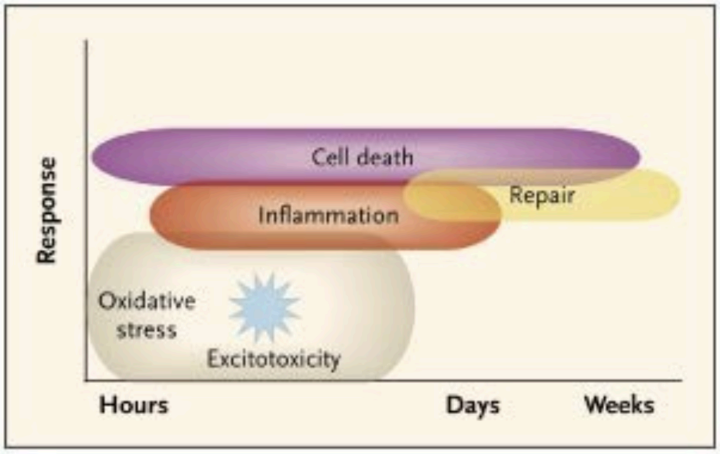
PART A: MANAGEMENT AND MONITORING OF NEONATES WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY

Neonatal encephalopathy (NE) is a clinical syndrome of disordered neonatal brain function occurring in the first days after birth [56]. In this work we will focus on neonates born beyond 35 weeks of gestation as they may be eligible for therapeutic hypothermia and as there early presenting signs is easier to recognize. Hypoxic-ischemic encephalopathy (HIE) or birth asphyxia is responsible for most, but not all, cases of neonatal encephalopathy [57]. Indeed, perinatal infections, genetic and epigenetic abnormalities, placental abnormalities, metabolic disorders, coagulopathies, maternal risk factors, and neonatal vascular stroke have also been implicated [58]. Given that the underlying nature of brain injury causing neurologic impairment in a newborn is often poorly understood, the term "neonatal encephalopathy" has emerged as the preferred term to describe the clinical syndrome of central nervous system dysfunction in the newborn period because it does not imply a specific underlying etiology or pathophysiology [59]. Neonatal encephalopathy has an incidence of 1.5 per 1000 live births in developed countries and up to 26 per 1000 live births in low-resource countries [60]. Worldwide, acute perinatal asphyxia is estimated to account for 23% of infant deaths.

1. MECHANISMS OF BRAIN INJURY IN HIE

After hypoxia-ischemia, the brain injury that occurs is commonly the result of both primary and secondary energy failure. The initial insult triggers a cascade of events, like the concept of falling dominos. During the initial phase, decreased cerebral blood flow leads to diminished oxygen and glucose energy substrates, decreased ATP production, and an increase in lactate levels. Lack of energy substrates results in a failure of Na^+/K^+ pumps and activation of an excitotoxic-oxidative cascade [61]. Continuation of this cascade leads to an altered cell membrane, impaired cellular integrity, and cellular apoptosis and necrosis. Once blood flow is restored, there is a brief period of normal cerebral metabolism, followed by the development of secondary energy failure. Continuation of the excitotoxic-oxidative cascade leads to secondary energy failure in the mitochondria. The lack of energy, the rise of inflammation, the altered growth factors, and the protein synthesis ultimately lead to brain cell apoptosis and necrosis over the next several days to weeks.

Figure 5: Mechanisms of Brain Injury in the Term Neonate [61]



2. RANDOMIZED CONTROL TRIALS OF THERAPEUTIC HYPOTHERMIA FOR NEUROPROTECTION IN HIE

Multiple randomized trials have shown that, when initiated within 6 hours of birth, either selective head cooling or whole-body cooling is associated with a reduction in mortality and long-term neurodevelopmental disability (NDD) for infants with moderate to severe HIE [62–65]. A systematic review and meta-analysis of 11 randomized trials including more than 1505 newborns with follow-up to at least 18 months showed that therapeutic hypothermia (TH) is associated with a significant risk reduction for death or moderate to severe neurodevelopmental disability (NDD) at 18 to 24 months (Relative Risk [RR] = 0.75; 95% confidence interval [CI] = 0.68–0.83), with the number needed to treat (NNT) of 7 (95% CI = 5–10) [66]. Furthermore, survival without NDD was also increased (RR = 1.63; 95% CI = 1.36–1.95).

When outcomes were analyzed based on the degree of encephalopathy at the time of randomization, pooled data from 5 trials showed infants with moderate encephalopathy who were treated with TH had a risk reduction for moderate to severe neurodevelopmental impairment or death of 0.68 (95% CI = 0.56–0.84) and a NNT of 6 (95% CI = 4–13) and those with severe encephalopathy had an RR of 0.82 (95% CI = 0.72–0.93) and a NNT of 7 (95% CI = 4–17). TH was shown to have a statistically and clinically significant effect on individual outcomes including cerebral palsy, neuromotor delay, and developmental delay.

The neuroprotective effect of TH carries through to early childhood. Following these results, therapeutic hypothermia (TH) has become the standard of care for neonates with moderate to severe neonatal encephalopathy secondary to birth asphyxia [67]. It is now a widely used treatment that has continued to be both safe and effective in improving neuro-developmental outcomes [66].

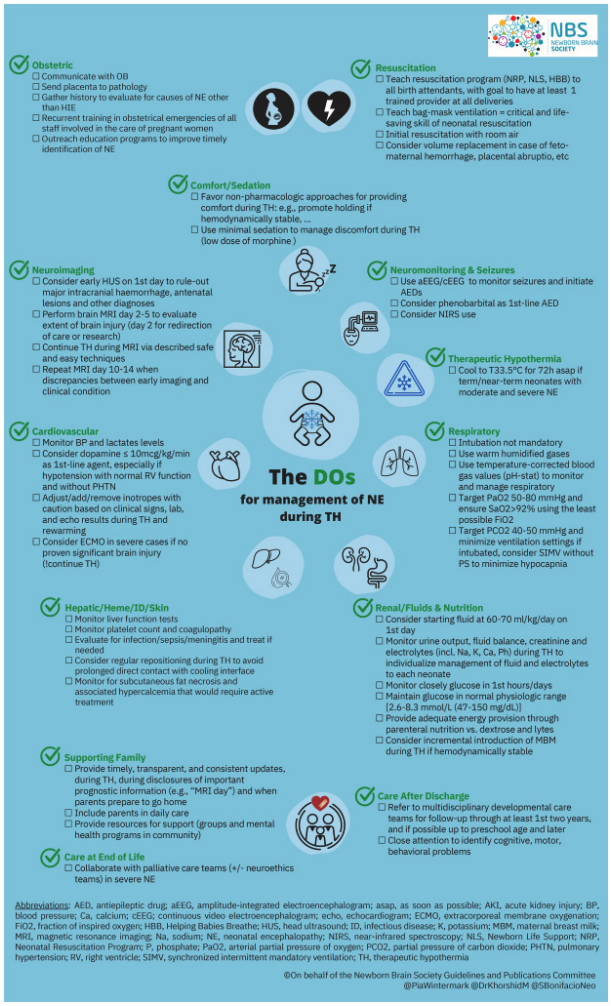
3. IMPLEMENTATION OF THERAPEUTIC HYPOTHERMIA

Therapeutic hypothermia consists in decreasing the infant's temperature to decrease the metabolic demand and reperfusion oxidative stress following an acute hypoxic event. Hypothermia should be started within the first six hours after delivery and continued for 72 hours at target temperature. The rectal temperature should be maintained at a target temperature of 33.5°C. Although direct comparisons are lacking, selective head cooling and whole-body cooling appear to have similar safety and effectiveness. However, whole-body cooling is preferred in most centers due to ease of administration. Therapeutic hypothermia is achieved by using a cooling device underneath or around the infant. Cooling can be started and maintained during neonatal transport if there is a need to transfer the infant to a specialized center. Hypothermia lasted for 72 hours in most of the cooling trials. However, one small trial stopped cooling between 48 and 72 hours for newborns who recovered neurologically [68]. A later randomized controlled trial of 364 neonates with HIE investigated if longer (120 hours) or deeper (32°C) hypothermia, or both, reduce early neonatal death or death or disability at age 18 months compared with hypothermia for 72 hours at 33.5°C [69,70]. This trial was stopped early for futility and evidence of increased mortality in the experimental (longer or deeper) cooling arms.

Therapeutic hypothermia is generally well-tolerated. Short-term adverse effects reported in randomized trials included sinus bradycardia, thrombocytopenia [66], and rarely subcutaneous fat necrosis, with or without hypercalcemia [71]. Parental separation and anxiety is an understudied significant side effect of therapeutic hypothermia [72].

Aside from treatment with therapeutic hypothermia, neonates with encephalopathy often require intensive multi-organ management. This includes treatment of seizures, adequate respiratory, cardiovascular, hematologic, infectious, and nutritional support, among others. Summary guidelines on the management of infants with encephalopathy presumed due to HIE treated with therapeutic hypothermia have been recently published and summarized in clinically useful care bundles (Figure 6) [73].

Figure 6: Care bundle for management of neonates with encephalopathy treated with TH



4. INDICATION OF THERAPEUTIC HYPOTHERMIA

Eligibility criteria for therapeutic hypothermia require the presence of 1) evidence of an acute perinatal event and 2) encephalopathy, in 3) an infant born at or after 36 weeks of gestation. Therapeutic hypothermia should be started as soon as possible and before 6 hours after birth.

Evidence of an acute perinatal event was defined differently in the major RCTs, and hence there is some variability between centers. One restrictive way to define the presence of an acute perinatal event is by having any of the following:

- Metabolic or mixed acidosis with a pH of ≤7.0 or a base deficit of ≥16 (sometimes down to 12 mmol/L) in a sample of umbilical cord blood or any blood obtained within the first hour after birth
- A 10-minute Apgar score of ≤5
- Ongoing resuscitation (e.g., assisted ventilation, chest compressions, or cardiac medications) initiated at birth and continued for at least 10 minutes, sometimes receiving CPAP may be enough.

Some centers use less stringent criteria and offer TH to neonates with milder acidosis and less need for respiratory support.

Evidence of moderate to severe encephalopathy on clinical examination between 1 and 6 hours also has varied definitions by centers and studies. The original clinical trials used different definitions of moderate to severe encephalopathy and there is significant practice variation as to how this criterion is defined.

Most centers use a modified Sarnat exam, with or without additional information on the presence of seizures. The modified Sarnat score includes 6 items: Level of consciousness, tone, posture, reflexes, activity, and autonomic function as illustrated in Table 3.

Table 3: Example of a modified Sarnat score used by the HEAL trial [74]

	Mild	**Moderate**	**Severe**
Level of consciousness	Hyperalert or irritable	Lethargic or poorly responsive	Minimal or no responsiveness
Spontaneous activity	Slightly decreased	Decreased	Absent
Posture	Mild distal flexion	Distal flexion complete extension	Decerebrate
Tone	Hypertonic	Hypotonic	Flaccid
Primitive reflexes **Suck** **Moro**	 Normal Low threshold	 Weak or bite Weak or incomplete	 Absent Absent
Autonomic **Pupils** **Resp**	 Equal, reactive Normal	 Constricted Periodic	 Dilated, sluggish or asymmetric No spontaneous

While TH has only been shown to improve outcomes in infants with moderate to severe encephalopathy, this treatment is increasingly used for neonates with mild encephalopathy. Studies suggest that newborns with mild encephalopathy can also exhibit brain injury [75,76]; however, the safety and efficacy of therapeutic hypothermia for mild encephalopathy has not yet been established in clinical trials [77,78].

PART B: PREDICTORS OF LATE SEIZURE OCCURENCE

Seizures are common in neonates with hypoxic-ischemic encephalopathy (HIE) [79–82] and are associated with short- and long-term unfavorable developmental outcomes [83] and an increased risk of moderate to severe injury on MRI [84,85]. Continuous cEEG monitoring is the gold standard for evaluating brain function and recording seizures. However, it is resource-intensive and requires a significant commitment in terms of equipment, time, and personnel [86]. Furthermore, continuous cEEG is not uniformly available in neonatal units [87]. Hence, amplitude-integrated EEG (aEEG) is routinely used in many units because it is easier to apply and interpret at the bedside by a neonatologist. aEEG has its own specific limitations, including having an interpreter-dependent sensitivity and specificity and a limited ability to detect short, focal, or low amplitude seizure [29]. In 2011, the ACNS recommended monitoring with continuous cEEG for at least 24 hours, all neonates at risk of seizure, including those with HIE [7]. Monitoring with either cEEG or aEEG during TH and rewarming is also recommended by the American Academy of Pediatrics, although the duration of monitoring is not specified [67].

Identifying neonates at higher risk of seizure is challenging, as no clinical variables - including mental status, clinically suspected seizures prior to monitoring, or biological signs (pH, base excess, or 10-minute Apgar score) - have been strongly associated with the occurrence of seizure during TH. The only variable associated with the development of seizure is an abnormal EEG background [82,88].

Seizures tend to arise within the first 24 hours following the acute insult [80,82]. Although rare, neonates with onset of seizures during rewarming have been described [82,84,89–91], prompting many neonatal units to monitor neonates undergoing TH for the entire duration of cooling and rewarming [80,81,92].

The aim of our study was to assess the prevalence of seizures in neonates with an initially normal or mildly abnormal background on continuous cEEG. We hypothesized that the number of neonates with seizure onset after 24 hours of a normal background with no seizure activity would be low. This would refine the guidelines for cEEG monitoring in neonates and help guide resource utilization and clinical management.

1. PATIENTS AND METHODS

Consecutive newborns with encephalopathy presumed secondary to HIE who underwent TH with whole-body cooling at University of California, San Francisco, over a 9-year period between April 2008 and April 2017 were considered for inclusion in this cohort study. According to this center guidelines, TH was provided to infants >36 weeks gestational age, with evidence of perinatal acidosis by one of the following: pH<7.0 or base deficit >12 on a cord or first patient blood gas, 10-minute Apgar score <5, or history of prolonged resuscitation at birth, and moderate to severe encephalopathy at any time within 6 hours of birth. To note, in our center as well as in most centers in the United States, EEG findings are not part of the inclusion criteria for TH. Exclusion criteria for TH included coagulopathy with active bleeding and prenatally diagnosed syndromes or metabolic disorders not compatible with life. Whole-body cooling was initiated as soon as possible using a servo-regulated cooling blanket device (Cincinnati Sub-Zero Blanketrol III). Target temperature of 33.5 °C was maintained for 72 hours from the time core rectal temperature was achieved, followed by gradual rewarming over 6-12 hours. Once screened as candidates for TH, infants born at outside hospitals were passively cooled. Most transports occurred under a passive cooling protocol with continuous rectal temperature monitoring, but more recently by active cooling at target temperature using a portable servo-regulated cooling blanket device (Tecotherm-Neo, Inspiration Medical LTD) [93]. All patients received morphine infusions (10-25 mcg/kg/h and boluses 5-10 mcg/kg as needed) throughout TH to reduce discomfort attributable to encephalopathy and to counteract the stress response induced by hypothermia and the shivering, which might reduce the effectiveness of hypothermia [62,94].

As part of our clinical protocol, continuous video-EEG (vEEG) was initiated as soon as possible after admission to the intensive care nursery using a Nicolet One vEEG system (April 2008 - Feb 2015) or Natus Networks vEEG system (February 2015-April 2017) and continued until rewarming was completed and at least for 24 hours after the last seizure. A trained technician applied surface electrodes according to the international 10–20 system, modified for neonates. Continuous vEEG recordings were interpreted by pediatric neurophysiologists for clinical use in the acute setting.

Using stored files and after being trained in neonatal EEG interpretation, we evaluated vEEG recordings of the first 24 hours. The EEG recordings were further reviewed by a neonatal neurophysiologist (blinded to all clinical factors except patient age and setting of TH), for the purpose of the study. EEG were classified based on the worst EEG grade that persisted for at least 1 hour, as previously

reported [79]. A normal cEEG was defined as a normal pattern for gestational age; a mildly abnormal cEEG was defined as an excessively discontinuous pattern with persistence of discontinuous activity occupying more than 50% of the recording and consisting of bursts of normal activity separated by inter-burst intervals > 6 and ≤ 15 seconds in duration, and amplitude between 5 and 25μV. Neonates with EEG background characterized by continuous low voltage, burst suppression pattern or low voltage undifferentiated were grouped together in a moderate to severe EEG background group (Figure 7).

Figure 7: Distinction between excessively discontinuous and burst suppression pattern on EEG

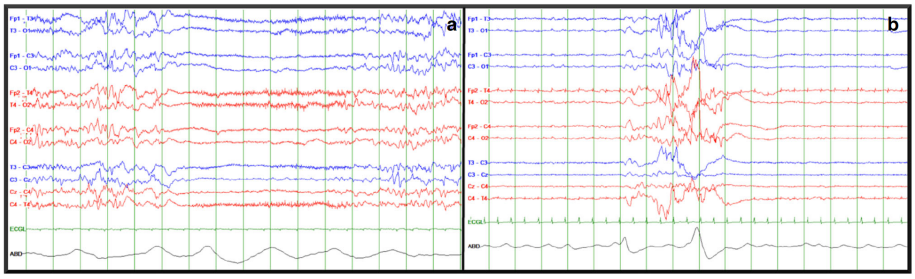


Fig. 1 Distinction between excessively discontinuous and burst suppression pattern on EEG. **a** Excessively discontinuous: burst of normal activity and amplitude, intermixed with interburst interval (IBI) lasting more than 6 s. **b** Burst suppression: short high-amplitude burst with no normal activity, in between prolonged period of very low amplitude. Note the electrocardiogram artifact, most evident in the Fp2-T4 leads. No discernible brain activity is recorded during the interburst interval.

A seizure was defined as a repetitive, evolving, and stereotyped EEG pattern, with a definite beginning and end, a minimum duration of 10 seconds, and a minimum amplitude of 2μV. Clinical seizures without cEEG correlate were not considered. Seizures were treated with antiseizure medication (ASM), including lorazepam, phenobarbital, fosphenytoin, and levetiracetam, according to institutional guidelines. Exclusion criteria for this study were: continuous cEEG unavailable for review or started after 24 hours of life, failure to complete 72 hours of TH, a moderate to severe cEEG background, and/or the presence of seizure in the first 24 hours of recording. The full duration of cEEG of the included neonates were reviewed until the end of recording to assess for background and the presence of seizures. Demographic data and MRI results were extracted from medical records. Infants were imaged shortly after rewarming using a specialized neonatal head coil. Imaging sequences included T1- and T2-weighted MRI and diffusion-weighted imaging. Brain injury was categorized as “none-mild” or “moderate-severe” as previously published [79,85]. The Committee on Human Research at UCSF approved this study.

2. RESULTS

During the study period, 331 newborns were treated with hypothermia, and 323 had continuous video-EEG available for review. Of these, 99 neonates (31%) were excluded because of an abnormal cEEG and/or seizure in the first 24 hours. Among them, 13 (4%) had seizures in the first 12 hours of recording in the context of a normal/mildly abnormal cEEG background, and 86 (27%) had a moderately to severely abnormal cEEG background. Two hundred twenty-four neonates (69%) had a normal/mildly abnormal cEEG background and no seizures in the first 24 hours of recording (Figure 8). Eight were rewarmed early (7 had severe PPHN, and one was rewarmed per parental request).

Figure 8: STROBE Flow diagram of observational study

The clinical characteristics of the 216 neonates included in the study are presented in Table 4.

Table 4: Clinical characteristics of 216 neonates with a normal/mildly abnormal background on cEEG in the first 24 hours after birth

Characteristics	
Male	107 (50)
Inborn	43 (20)
Emergent cesarean	110 (51)
Gestational age, in weeks	39.3 ± 1.5
10-min Apgar (n = 193)	6 ± 2
First pH (n = 213)	7.07 ± 0.18
First BE (n = 209)	−15.6 ± 5.2
Age at therapeutic temperature, in hours (n = 208)	5.1 ± 2.6

Data are presented as n (%) or mean ± SD

Continuous cEEG was started at a median age of 8.2 hours (interquartile range 5.7 to 10.6 hours of life) and continued for a median of 87 hours (interquartile range 79 to 94 hours). No patients with an initially normal/excessively discontinuous EEG evolved into a more severe pattern afterwards and no seizures were detected in this large cohort. Hence, the probability of seizure occurrence after 24 hours of a normal/mildly abnormal cEEG calculated from our cohort is extremely low (0%; 95% confidence interval 0-1.38%).

Interestingly, 21 (10%) received ASM prior to cEEG recording for paroxysmal events that were presumed to be and treated as seizures based on clinical

observation. Those patients were treated with phenobarbital (n=13), lorazepam (n=5), or both (n=3). None of these 21 neonates had subsequently EEG-confirmed seizures during the monitoring.

214/216 patients had MRI performed after TH (median: 4 days of life). Of those, 208 (97%) had no to mild brain injury.

3. DISCUSSION

In this large single-center study of neonates continuously monitored with video-EEG during TH and rewarming, patients with a persistently normal or mildly abnormal cEEG background who did not experience seizures in the first 24 hours, did not develop seizures during the rest of monitoring. Furthermore, neonates with normal/mildly abnormal EEG background from the beginning had overall a low risk for seizures (13/229; 5.7%) and in all cases, seizures occurred within the first 12 hours of recording, corresponding to the first 24 hours of life. The low incidence of seizures in neonates with a normal or mildly abnormal EEG background is in keeping with previous studies. In 1998, Laroia *et al.* demonstrated that in neonates at risk of seizures, early EEG background predicts seizure occurrence (only one of 24 neonates with a normal or mildly abnormal EEG background had seizure compared to 22/25 with an abnormal background) [88]. In the cohort reported by Del Balzo et al., of 20 neonates undergoing TH, none of the ten neonates with a normal EEG background had seizures [95]. In contrast, in a recent multi-center study, a third of neonates (18/54) with a normal/excessively discontinuous background had seizures [82]; all occurred early, except for one who had seizure onset during rewarming. Seizure onset after 24 hours of EEG monitoring is reported in the literature [81,82,84,91,96,97]. Information on EEG background in those cases is scarce, although most reported neonates had severe encephalopathy. Some studies have included in the definition of excessively discontinuous pattern also more severe patterns such as burst-suppression and longer interburst interval [98,99]. This could explain the higher percentage of seizures and the poor outcome reported for those patients.

In our cohort, 69% of neonates had a normal or mildly abnormal EEG and no seizures in the first 24 hours of recording, and only 31% had a moderately to severely abnormal EEG defined as either presence of seizure in the context of a normal/mild background or a moderate/severe background. This proportion of neonates with normal or mildly abnormal EEG is higher than previously reported in neonates treated with TH [81,100]. Possible explanations include a drift toward cooling neonates with milder encephalopathy as described in other cohorts [101,102], earlier recognition of encephalopathy, and faster implementation of

cooling leading to the possibility that, by the time EEG is started, the brain activity has already improved in some neonates. Furthermore, our definition of excessively discontinuous background encompasses a wide range of interburst intervals (up to 15 seconds). In fact, in the setting of TH, an excessively discontinuous cEEG pattern is not infrequently seen, possibly due to the effects of concomitant medications such as morphine [103] and/or the decreased temperature itself. This could explain why, even in the presence of an excessively discontinuous background on cEEG, no neonates had seizure onset after 24 hours. Furthermore, consistent with other studies [79,104], we did not observe evolution from normal/excessively discontinuous background into more severe patterns. In the context of TH for presumed HIE, EEG background appears to have the greatest prognostic value at 24 hours of monitoring for later seizure occurrence following presumed HIE [79,100,105].

In our large cohort, most neonates with a normal/excessively discontinuous EEG background (97%) had no or mild MRI brain injury. The present data are in line with previous studies showing that 100% of neonates with an early normal background and 73-75% of those with an excessively discontinuous pattern after rewarming had no or only mild MRI injury [79,100]. This association between a normal/mildly abnormal initial EEG and none or mild MRI brain injury supports the observation that an excessively discontinuous background in the setting of TH is usually not associated with a poor outcome.

Lately, many centers have started offering TH to neonates with mild encephalopathy [101,102], despite lack of data in support of this practice. This changing attitude has been explained by many arguments including reports of adverse neurodevelopmental outcome in neonates with mild hypoxic-ischemic encephalopathy (HIE) [106,107], difficulty in grading neonatal encephalopathy soon after birth and concern about missing the 6-hour window for initiating cooling [101]. Since the clinical evolution of the encephalopathy can be rapid, it is possible that in our cohort some neonates who were cooled had only a mild encephalopathy.

Because none of the neonates without seizure activity and a normal/mildly abnormal EEG during the first 24 hours subsequently developed seizures, it may be reasonable to discontinue cEEG monitoring at that time and in that context. Our finding could help refine the guidelines for cEEG monitoring in patients with HIE. Although cEEG monitoring is the gold standard for detecting seizures, it is labor-intensive as trained technologists and physicians are required to review traces, often at all hours of the day and night [86]. Discontinuing cEEG in selected patients after 24 hours may lead to better allocation of resources. This recommendation should be balanced with the risk that, without EEG monitoring,

some neonates may be diagnosed with seizures based on suspicious movements, exposing them to unnecessary ASM treatment [92]. The role of aEEG for seizure screening and monitoring of background evolution (including return to sleep wake cycling) merits additional study.

To our knowledge, this is the largest reported cohort of neonates with HIE treated with hypothermia and continuously monitored with video-EEG. Nevertheless, our study has some limitations. First, background assessment was performed by visual interpretation of a single rater. Although a single EEG rater provide consistency, this approach may limit the generalizability of the findings. A recent study showed that the interrater agreement for classification of cEEG background comparing 3 raters was 72% full agreement [108]. More accurate ways of classifying cEEG background in neonates, including automated quantitative analysis of EEG activity may help in overcoming those challenges and provide objective basis for studies across all centers worldwide [109,110]. Additionally, 10% of neonates in our cohort had received ASM prior to cEEG recording for paroxysmal events that were presumed to be seizures on a clinical basis. The significance of these events remains unknown, but ASM might have protected them from developing further seizures in the next hours or days, biasing our results toward a lower seizure incidence. However, in previous studies, ASM administration prior to monitoring was not associated with a lower or higher EEG-proven seizure risk [82]. This may be due to bias inherent to observational data as neonates at the highest risk of seizures were more likely to be given ASM before transport. In addition, many movements concerning for seizures are not epileptic, and ASM would not be indicated in those cases.

4. CONCLUSIONS

Our data demonstrate that the risk of developing seizures is extremely low in infants with encephalopathy presumed due to HIE treated with TH with a normal/mildly abnormal continuous cEEG and no seizure activity during the first 24 hours. Furthermore, none of these infants evolved into a more severe pattern and very few have evidence of moderate to severe brain injury on MRI.

We suggest that cEEG may be safely discontinued after 24 hours of normal recording in neonates who are treated with therapeutic hypothermia. This practice could lead to better resource allocation focused on higher risk and more fragile infants. Discontinuation of cEEG after 24 hours in HIE patients with a very low risk of seizures would optimize the utilization of resources and workload without compromising seizure detection.

The application and interpretation of neonatal EEG remains a challenge for many centers. This highlights the necessity to train local pediatrics and obstetric teams in the recognition of the signs of encephalopathy, to develop efficient and skilled transport teams who can bring neonates with encephalopathy in neonatology unit with neurological expertise, and to build centers of excellence with a multidisciplinary approach where neonates with seizures or encephalopathy should benefit from the best possible care to maximize the chance of positive outcomes.

CHAPTER 3: THE ELECTRO-CLINICAL SPECTRUM OF NEONATAL-ONSET EPILEPSIES

PART A: REVIEW OF THE DIFFERENT ETIOLOGIES AND KNOWN PRESENTATION OF NEONATAL-ONSET EPILEPSIES

While most seizures in the neonatal period are due to acute brain injury, a substantial proportion of them reflects the onset of epilepsy [4,6]. Neonatal-onset epilepsies have historically been lumped together within the larger group of seizures due to acute brain injury. As described in the first chapter of this thesis, our understanding of neonatal-onset epilepsies has greatly improved in the last decade thanks to increased use of EEG monitoring, neuroimaging, metabolic, and genetic testing in the Neonatal Intensive Care Unit (NICU). Advances in genomic technologies have unveiled several pathogenic variants causing neurodevelopmental disorders, including epilepsies [111–113]. In addition, the role of clinical phenotype combined with genetic testing as a guide for short and long-term management with the potential to improve outcomes is expanding [8,114], leading to a new precision medicine approach.

Early recognition of the electro-clinical phenotypes specific to each condition is essential for targeted treatment. The recommendations of the ACNS in 2011 to monitor all neonates at high risk for seizures with long-term video-EEG [7], as well as the development of brain-oriented neonatal ICUs, has led to enhanced collaboration between neonatologists and neurologists resulting, for the first time, in the delineation of specific electro-clinical phenotypes based on etiology [8–10].

The first neonatal-onset epilepsy syndromes to be recognized were the Benign Familial Neonatal Epilepsy (BFNE), Ohtahara syndrome (OS), and Early Myoclonic Encephalopathy (EME) [11,12]. While BFNE is a self-limited form of epilepsy of the newborn, associated in most cases with normal development, EME and OS are characterized by severe disruption of cerebral functions associated with seizures, often intractable. EME and OS are also known as neonatal epileptic encephalopathies with suppression-burst as they share the important features of a burst-suppression pattern on EEG associated with clinical signs of encephalopathy [115]. EME is characterized by erratic, fragmentary myoclonus with the eventual development of focal seizures and epileptic spasms. EME is most often due to a metabolic disease including glycine encephalopathy, propionic acidemia, D-glyceric acidemia, and methylmalonic aciduria [116]. Numerous cases of pyridoxine dependency presenting as EME have been reported, supporting an early therapeutic trial with pyridoxine in this condition [117]. On the other hand, Ohtahara syndrome consists of tonic spasms, isolated or in clusters, symmetric or asymmetric, occurring during both wakefulness and sleep. Partial seizures may also be present. Structural brain abnormalities have been frequently reported associated with OS. More recently, *ARX* and *STXBP1* have

been implicated in OS, as well as other genes, including *CDKL5*, *KCNQ2*, and *KCNT1* [118,119]. However, further delineation of their electro-clinical phenotypes has suggested that those do differ from the original description of OS. Although they share with OS age at onset, developmental delay, and seizure intractability, they represent more specific, discrete etiology-related syndromes [8,120,121].

The last International League Against Epilepsy (ILAE) position paper on the classification of the epilepsies [53] introduced the new concept of developmental and epileptic encephalopathies (DEE), which is particularly relevant for genetic epilepsies with onset in the neonatal period. Epileptic encephalopathies are conditions in which abundant epileptiform activity interferes with development, resulting in cognitive slowing, plateauing, or regression [3]. The concept of epileptic encephalopathy entails that the epileptic activity itself contributes to the poor neurologic outcome above and beyond what might be expected from the underlying pathology alone. The implication is that amelioration of the epileptiform activity may improve the developmental consequences of the disorder. However, in some neonatal epilepsies, such as *KCNQ2* or *STXBP1* encephalopathy, the disease *per se* may directly lead to both severe epilepsy and profound intellectual disability as two independent dimensions of the phenotype. The developmental consequences may arise more from the direct effect of the genetic mutation than from the effect of the frequent epileptic activity on development. The relevance of these two components, the epileptic and the developmental component, can vary in neonatal-onset epilepsies. For instance, in some patients with *KCNQ2* or *STXBP1* encephalopathy, the epilepsy may settle down relatively early, but the developmental consequences may remain profound. These concepts are crucial to understand the disease processes for both families and clinicians [53].

1. CLASSIFICATION OF NEONATAL-ONSET EPILEPSIES

As discussed in chapter 1, the most recent ILAE classification of the epilepsies incorporates etiology along each step of diagnosis. Etiology was broken into six subcategories: Structural, genetic, infectious, metabolic, immune, and unknown [53]. For some patients, two or more subcategories may account for the epilepsy's etiology, and the importance of the etiological group may depend on the circumstances. Indeed, a neonate with pyridoxine-dependent epilepsy has both a metabolic and a genetic epilepsy. The metabolic part is important for targeted treatment, while the genetic element is essential as a transmission risk factor.

Therefore, neonatal-onset genetic epilepsies can be classified into three large groups: Genetic conditions associated with structural brain anomaly, genetic

conditions associated with metabolic changes, and genetic conditions resulting in functional cortical anomalies (Table 5).

Table 5: Examples of neonatal-onset epilepsies associated with monogenic variants

Group	Subgroup	Examples
Structural	Cell proliferation or apoptosis disorders	Focal cortical dysplasia & Dysplastic megalencephaly (*AKT3, TSC2, MTOR*)
	Cell migration disorders	Heterotopia (*FLNA, ARFGEF2*) Lissencephaly (*TUBA1A, LIS1*)
	Post-migration disorders	Polymicrogyria & Schizencephaly (*MTOR, WDR62*)
Metabolic	Inborn errors of metabolism in which epilepsy dominates the clinical presentation	Pyridoxine dependent epilepsy (ALDH7A1) Sulfite oxidase deficiency (SUOX) Molybdenum cofactor deficiency (*MOCS1)* Glycine encephalopathy (*SLDC*)
Functional	Channelopathies	Benign Familial Neonatal Epilepsy (KCNQ2/3) *KCNQ2* encephalopathy Epilepsy of infancy with migrating seizures (*KCNT1*)
	Cell signaling disorders	*CDKL5* encephalopathy Lethal neonatal rigidity and multifocal seizures (*BRAT1*)
	Synaptic transmission disorders	*STXBP1* encephalopathy

This table is intended to give some examples within the classification of neonatal-onset epilepsies and is not an exhaustive list. Some genes are associated with multiple pathophysiologic mechanisms and phenotypic expressions (genetic heterogeneity). Some phenotypes are associated with multiple genes (phenotypic heterogeneity).

This classification will likely be refined over time as our understanding of the physiopathology underlying each condition improves.

1.A. GENETIC CONDITIONS ASSOCIATED WITH A STRUCTURAL BRAIN ANOMALY

In these diseases, genetic mutations can either lead to disruption of the cortical development, or predispose the brain to injury (e.g., hemorrhage, stroke). An increasing number of genes associated with malformations of the cortical development have been identified [122], some presenting with severe intractable epilepsy in the neonatal period. Our understanding of the genetic and molecular

pathways underlying these disorders is rapidly evolving. Malformation of the cortical development can be due to:

1) disorders of the cell proliferation or apoptosis which includes primary microcephalies and overgrowth disorders (e.g., focal cortical dysplasia, dysplastic megalencephalies)
2) disorders of cell migration (e.g., heterotopias, lissencephaly, cobblestone malformations)
3) disorders of the post-migrational development (e.g., polymicrogyria, schizencephaly).

These mechanisms often overlap making an accurate unique classification system challenging [123,124]. All these cortical malformations have been associated with seizures in the neonatal period. The mode of transmission is variable. Some individuals carry systemic genetic mutations while in others, such as neonates with postzygotic mutations (mosaicism), analysis of brain tissue itself can provide the clue [123].

Early identification of neonates with epilepsy associated with cortical malformations is important as they may benefit from early surgery [125] or treatment with vigabatrin [126].

1.B. GENETIC CONDITIONS ASSOCIATED WITH INBORN ERRORS OF THE METABOLISM

Neonates with inborn errors of metabolism can present with seizures. Depending on the primary disease, the pathophysiologic mechanism leading to seizure can be:

1) An excess of a neurotransmitter with excitatory action, or a deficiency of a neurotransmitter with inhibitory action (e.g., deficiency of pyridoxine causes a deficiency of GABA in pyridoxine-dependent seizures);

2) A deficient brain energy substrate (e.g., GLUT-1 deficiency syndrome);

3) A defect in brain structure produced by the inherited metabolic disorder in utero that results in various types of malformation (e.g., peroxisomal disorders);

4) An acute brain lesion caused by inherited metabolic disorder (e.g., mitochondrial encephalopathies);

5) An endogenous intoxication due to the inherited metabolic disorder (e.g., aminoacidopathies such as glycine encephalopathy).

Early recognition of inborn errors of metabolism is essential as traditional anti-seizure medications are usually ineffective. Appropriate treatment, targeted to

each condition, may prevent neurological deterioration and long-term sequelae [127].

A high index of suspicion is warranted in the context of seizures occurring after an initial symptom-free period followed by feeding difficulty, lethargy or extreme irritability, and respiratory distress. Abnormal biochemical findings such as high anion gap metabolic acidosis, ketonuria, or hyperammonemia, as well as dysmorphic features, liver disease, cardiomyopathy, cataracts, hearing loss, and renal tubular defects can provide clues to the diagnosis [128].

Some inborn errors of metabolism present with seizures as the main symptom. The best examples are pyridoxine-dependent epilepsy, sulfite oxidase deficiency, and molybdenum cofactor deficiency. These disorders generally do not present with biochemical abnormalities detected through routine laboratory testing, with a few exceptions including Molybdenum cofactor deficiency in which simple measurement of plasma uric acid level is highly suggestive, and diagnosis requires specific metabolic evaluation. They should be suspected in patients with very early, even antenatal, seizures refractory to conventional therapies with progressive worsening of clinical and electroencephalographic abnormalities, including burst suppression or hypsarrhythmia pattern on EEG, and/or evidence of severe injury on MRI without any obvious hypoxic insult at birth [128].

1.C. GENETIC CONDITIONS ASSOCIATED WITH FUNCTIONAL ANOMALIES

The term “genetic epilepsies” includes all epilepsies where “epilepsy is, as best as understood, the direct result of a known or presumed genetic defect, in which seizures are the core symptom of the disorder” as defined in the new terminology of the ILAE [3].

Hence, this excludes epilepsies where a distinct structural or metabolic condition has been demonstrated to be associated with a substantially increased risk of developing epilepsy. It includes channelopathies (e.g., *KCNQ2, KCNT1*), disorders of the cell signaling (e.g., *CDKL5, BRAT1*) and disorders of the synaptic transmission (e.g., *STXBP1*). In this group of neonatal-onset epilepsies, the imaging findings are usually non-specific, ranging from normal brain to microcephaly, diffuse hypomyelination, thin corpus callosum [129], or showing transient hyperintensities in the basal ganglia, thalami, or hippocampus [130,131].

2. PLACE OF GENETICS TESTING IN NEONATAL-ONSET EPILEPSIES

Although most neonatal seizures are due to an acute brain injury (e.g. HIE, stroke, infection or transient metabolic disturbance), around 13% of neonates with seizures have a neonatal-onset epilepsy [4] with a suspected or identified genetic cause. Sometimes, neonates with early-onset epilepsies can present with a clinical picture of acute seizures [6]. This could be explained by the fact that their genetic condition predisposes them to a poor neonatal adaptation.

Recent studies have demonstrated the central place of genetic testing in the diagnosis and management of neonatal-onset epilepsies. Depending on inclusion criteria, the diagnostic yield of genetic testing in neonates with epileptic encephalopathy ranges from 35 to 80% [6,47,132–134]. Genetic diagnosis is essential as it provides important prognostic and genetic information for family planning and patient care, and it prevents unnecessary diagnostic testing. Given the high diagnostic yield and management impact, genetic testing should be considered as early as possible, especially in certain epilepsy phenotypes [135].

Depending on phenotype, family history and suspected etiology, different types of genetic testing may be appropriate. We detailed in chapter 1 the different types of genetic tests available, their strengths and their limitations. As a reminder, chromosomal microarray evaluates for large deletions or duplications of segments of DNA (e.g., copy number variants). In contrast, whole exome sequencing allows for the analysis of DNA at the single-base level. Using a set of techniques known as next-generation sequencing, the coding portion of the genome (exome), representing about 1.5% of the genome, is evaluated. However, with these techniques, coverage is uneven across genes and parts of genes [44], and these techniques cannot detect copy number variants (CNV).

In addition, copy number variants, too small to be detected by standard chromosomal microarray, may not be detected on whole exome sequencing either. Gene panels combine next-generation sequencing of select genes, supplemented with Sanger sequencing and hybridization approaches. This ensures excellent coverage of the included genes, as well as detection of smaller deletions and duplications not detected by other approaches. However, the number of genes included in a panel is limited, and the diagnosis will be missed if the responsible gene is not included. We will show in part C why this is essential as, for example, *BRAT1* gene was not included in neonatal-infantile epilepsy panels in 2020, leading to a negative panel in an infant with *BRAT1* associated epileptic encephalopathy.

In general, if the phenotype is distinctive for one or a small number of genes, it is wiser to start with a gene panel that includes the suspected genes, followed by whole exome sequencing if the panel results are inconclusive [40].

2.A. MODE OF INHERITANCE

A genetic mutation can be suspected when there is a suggestive family history (e.g., BFNE associated with autosomal dominant *KCNQ2/3* pathogenic variants). However, the presence of genetic epilepsy does not equate to inherited. Many genetic epilepsies, particularly the severe ones, result from *de novo* mutations. Some individuals have mosaicism for a specific genetic variant. The number and type of cells carrying the mutation will affect the severity of the disease and the transmission risk. This has been shown in patients with mosaicism for *SCN1A* and *KCNQ2* variants [136,137].

Sometimes, epilepsy follows complex inheritance, which implies multiple genes with or without an environmental contribution. Susceptibility variants may be identified that contribute to causation but are insufficient alone to cause epilepsy. In this setting, there may be no family history of seizures, because other family members do not have enough epilepsy-associated genetic variants to be affected.

2.B. GENOTYPE-PHENOTYPE CORRELATION

The semiology of seizures in neonatal-onset epilepsies is still poorly defined as most cases are diagnosed retrospectively, and accurate documentation of seizures at presentation is often unavailable. In recent years, the wide use of video-EEG in neonates with seizures, together with an increased awareness for these rare disorders, has improved the recognition of distinct electro-clinical etiology-specific phenotypes.

This is the case for *KCNQ2*-related epilepsy that typically presents with seizures alternating laterality, characterized by initial asymmetric tonic posturing, often with apnea and desaturation, at times followed by a unilateral or bilateral clonic component [8–10]. Recognition of this seizure semiology in the appropriate clinical setting leads to the early treatment with sodium channel blockers, including carbamazepine or oxcarbazepine [9], even before genetic tests results become available. Other pathognomonic electro-clinical phenotypes include the association of epilepsy of infancy with migrating focal seizures with *KCNT1* encephalopathy [111] and the classical Ohtahara phenotype with burst-suppression and spasms associated with *STXBP1* encephalopathy [115,138].

Despite these well-described electro-clinical phenotypes associated with specific genetic mutations, many epileptic syndromes and electro-clinical phenotypes

have been associated with multiple genes (genetic heterogeneity). For example, BFNE is most often associated with *KCNQ2* variants but can also be due to *KCNQ3* mutations despite the same electro-clinical presentation.

On the other hand, most genes are associated with a spectrum of phenotypes (phenotypic heterogeneity). For example, variants in *KCNQ2* or *SCN2A* genes have been identified in patients with DEE and patients with self-limited disease. Understanding the phenotypic spectrum associated with mutations of a specific gene is essential. Finding a mutation in a specific gene does not, on its own, enable prediction of the treatment response or outcome, and interpretation of its significance needs to be considered in the context of the electro-clinical presentation. For instance, more than the genetic diagnosis, the EEG background can help in discriminating neonates with benign epilepsy (well-organized interictal brain activity) from those with severe epilepsy (profoundly abnormal EEG background) in patients with *KCNQ2* mutations. However, there are conditions such as *CDKL5* and *SLC13A5* that can initially present with a normal interictal EEG that deteriorates over time [139,140]. Hence, the initial normality of EEG in this context is not predictive of a good outcome.

Genetic information should be used as one piece of a puzzle. Together with clinical presentation, seizure semiology, interictal and ictal EEG findings, and MRI findings, they will help the clinician in management decisions and outcome prediction.

Table 6 describes the most common electro-clinical neonatal presentation of common genetic mutations. Although some of those genes have been associated with a spectrum of phenotypes, sometimes with later onset, only the forms with neonatal-onset are described in this table.

Table 6: Distinctive features of various neonatal-onset epilepsies associated with single gene mutations.

Phenotype MIM number Transmission	Gene/locus MIM number	Seizures	Interictal	Treatment	Outcome Comments
Pyridoxine-dependent epilepsy **#266100** **AR**	*ALDH7A1* 107323	Semiology: multifocal clonic seizures, myoclonic jerks, rarely tonic seizures Frequency: high, up to SE	Encephalopathy EEG: diffuse bursts of 1–4 Hz sharp and slow activity, if untreated can progress into BS	Pyridoxine, lysine restricted diet, L-arginine supplements	ID, motor and/or speech delay in up to 75% Treat at risk pregnant women and neonates
Pyridoxamine 5-phosphate oxidase deficiency **#610090** **AR**	*PNPO* 603287	Semiology: clonic seizure + myoclonic jerks +/– tonic seizure Frequency: high, up to SE	Encephalopathy, may mimic HIE EEG: diffuse bursts of 1–4 Hz sharp and slow activity, if untreated can progress into BS	Pyridoxal phosphate (some may respond to pyridoxine)	From mostly normal to severe ID and death
Glycine encephalopathy **#605899** **AR**	*GLDC* 238300 *AMC* *GCSH*	Semiology: myoclonic or multifocal clonic seizures Refractory	Normal at birth, progressive encephalopathy in the first hours Frequent myoclonus and hiccup EEG: BS pattern, often asynchronous	No effective treatment	Apnea and early death; moderate to profound ID in survivors.
Sulfite oxidase deficiency **#272300** **AR**	*SUOX* 606887	Semiology: myoclonic or multifocal clonic seizures Frequent and refractory	Severe encephalopathy, may mimic HIE EEG: BS pattern	No effective treatment	Profound ID, movement disorder and early death
Molybdenum cofactor deficiency **#252150** **AR**	*MOCS1* 603707 *MOSC2* *GPHN*	Semiology: myoclonic or multifocal clonic seizures Frequent and refractory	Encephalopathy, exaggerated startle reaction, axial hypotonia, limb hypertonia	Daily cyclic pyranopterin before seizure onset	Profound ID, early death

Phenotype MIM number Transmission	Gene/locus MIM number	Seizures	Interictal	Treatment	Outcome Comments
***SLC13A5*-associated EE #615905 AR**	*SLC13A5* 608305	Semiology: focal seizures,automatisms, clonic seizures, and desaturation Refractory seizure leading to SE	Good perinatal adaptation evolves to encephalopathy. EEG: normal/mildly discontinuous background initially MRI: punctuate white matter lesions	Lidocaine, midazolam and ketogenic diet effective in some patients	Severe to profound ID, frequent choreoathetosis, ataxia, dystonia
Benign familial neonatal epilepsy (BFNE) #121200 #121201 AD	*KCNQ2* 602235 *KCNQ3* 602232	Semiology: asymmetric tonic posturing shifting laterality +/– clonic movement, often with apnea and desaturations Frequency: variable up to SE aEEG: sudden lower and upper margin rise followed by amplitude depression	Normal clinical exam between seizures EEG: normal background	Carbamazepine, oxcarbazepine	Normal; later epilepsy in up to 25%
***KCNQ2*-EE #613720 AD**	*KCNQ2* 602235	Semiology: asymmetric tonic posturing shifting laterality +/– clonic movement with apnea and desaturations Frequency: up to 35 seizures/hour	Encephalopathy EEG: lack of organization and multifocal epileptiform abnormalities	Carbamazepine, oxcarbazepine, phenytoin	Severe ID despite good seizure control

Phenotype MIM number Transmission	Gene/locus MIM number	Seizures	Interictal	Treatment	Outcome Comments
Benign familial neonatal–infantile epilepsy (BFNIE) **#607745** **AD**	*SCN2A* 182390	Semiology: cluster of focal clonic or focal tonic seizures Frequency: seizures resolve by 12 months	Normal clinical exam between seizures EEG: normal background	Carbamazepine, oxcarbazepine, phenytoin, lidocaine	Normal development Low risk of seizure recurrence
***SCN2A*-EE** **#613721** **AD**	*SCN2A* 182390	Semiology: cluster of focal seizures with tonic component	Encephalopathy EEG: multifocal spikes or BS pattern	Carbamazepine, phenytoin	Severe ID, hypotonia, movement disorder
***CDKL5*-EE** **#300672** **X-linked dominant**	*CDKL5* 300203	Semiology and frequency: tonic seizures, epileptic spasms; typically, "hypermotor–tonic–spasm" sequence	Encephalopathy EEG: background initially normal than deteriorates	No effective treatment	Moderate to severe ID Mostly in girls; boys have severe phenotypes
KCNT1-EE **#614959** **AD**	*KCNT1* 608167	Semiology and frequency: sporadic focal seizure +/– autonomic symptoms → frequent clusters of refractory prolonged multifocal seizures	No encephalopathy at birth, evolves to severe hypotonia and encephalopathy EEG: multifocal bilateral paroxysmal event with migrating features	Bromide, levetriacetam. Quinidine (inconsistent efficacy)	Severe to profound ID despite seizure control Most common cause of epilepsy of infancy with migrating focal seizure
***STXBP1*-EE** **#612264** **AD**	*STXBP1* 602926	Semiology: epileptic spasms, often associated with focal seizures Refractory	Encephalopathy EEG: BS pattern	No effective treatment	Moderate-to-severe ID, ataxic gait and paroxysmal movement Common cause of OS

Phenotype MIM number Transmission	Gene/locus MIM number	Seizures	Interictal	Treatment	Outcome Comments
Lethal neonatal rigidity and multifocal seizures #614498 AR	*BRAT1* 614506	Semiology: multifocal myoclonic seizures, apnea and subclinical seizures	Microcephaly, non-epileptic myoclonic jerks, severe hypertonia and autonomic dysfunction EEG: lack of detailed information	No effective treatment	Autonomic instability leading to early death in the first year of life

Semiology: Most frequent semiology described at seizure onset. AR: Autosomal recessive; AD: Autosomal dominant; BS: Burst suppression ; EE: Epileptic encephalopathy ; HIE: Hypoxic-ischemic encephalopathy ; ID: Intellectual disability; OS: Ohtahara syndrome; SE: Status epilepticus.

3. ETIOLOGY-SPECIFIC NEONATAL-ONSET EPILEPSIES

3.A. INBORN ERRORS OF METABOLISM ASSOCIATED WITH ISOLATED SEIZURES OR EPILEPTIC ENCEPHALOPATHY

PYRIDOXINE-DEPENDENT EPILEPSY

Pyridoxine-dependent epilepsy is associated with mutations in *ALDH7A1* gene, which encodes antiquitin, a critical enzyme for cerebral lysine catabolism. In the typical presentation, seizures arise in the first days of life, sometimes even in utero. Multifocal clonic seizures are present in most neonates, often accompanied by multifocal myoclonic jerks and rarely by tonic seizures. Seizures tend to be frequent, often leading to status epilepticus [141]. The phenotypic spectrum of pyridoxine-dependent epilepsy is broad. Fetal ventriculomegaly and multisystemic dysfunction in the neonatal period have been described. Later seizure onset, autistic features, and a myriad of neurological and systemic symptoms occurring outside the neonatal period have also been reported and are out of the scope of this review [142]. There is no EEG pattern strictly associated with pyridoxine-dependent epilepsy. However, in some neonates, an EEG pattern of generalized bursts of 1-4 Hz sharp and slow activity, or burst-suppression, particularly in untreated cases, has been described [143,144]. Structural brain abnormalities may co-exist [117]. Urinary l-α-aminoadipic semialdehyde concentration determined by liquid chromatography tandem mass spectrometry can be used as a screening test with confirmation by DNA sequencing of the *ALDH7A1* gene.

A trial of pyridoxine, 100-300 mg IV, ideally during EEG monitoring, should be given as soon as the diagnosis is suspected. This should be done in a setting where respiratory support can be provided, as apnea has been reported in responders. Typically, in case of response, seizures cease, and EEG normalizes over 24 to 48 hours. However, responsiveness to pyridoxine is not confirmatory of the condition, and absence of a response does not rule out the diagnosis [145]. Seventy-five percent of affected individuals have neurodevelopmental challenges (intellectual disability, motor and speech delay), even despite early treatment and good seizure control with pyridoxine [146]. Recently, the combination of pyridoxine with a lysine-restricted diet and L-arginine supplements has shown some promise in reducing toxic metabolites in the brain and possibly improving outcome [147]. In at-risk families, antenatal and prophylactic treatment is recommended as it may prevent intrauterine seizures and improve neurodevelopmental outcomes [148,149]. However, even with effective antenatal treatment and absence of postnatal seizures, affected patients with poor cognitive outcomes have been

reported [150]. Diagnosis should be confirmed as early as possible to avoid potential side effects of high dose pyridoxine in unaffected neonates [145,151].

Pyridoxine 5-phosphate oxidase deficiency, a much rarer entity, can present similar clinical and electrophysiological findings as pyridoxine-dependent epilepsy. Patients are responsive to pyridoxal phosphate or sometimes to pyridoxine. Genetic testing is needed to confirm the diagnosis [152].

GLYCINE ENCEPHALOPATHY

Glycine encephalopathy (also known as nonketotic hyperglycinemia) is one of the main causes of early myoclonic epilepsy. It is an autosomal recessive disorder of the glycine metabolism associated with deficient activity of the glycine cleavage enzyme system leading to accumulation of large quantities of glycine in all body tissues, including the brain. The glycine cleavage enzyme system consists of 4 proteins: glycine decarboxylase (P-protein), aminomethyltransferase (T-protein), hydrogen carrier protein (H-protein), and dihydrolipoamide. Pathogenic variants in P and T proteins, respectively encoded by *GLDC* and *AMT,* are the major causes of glycine encephalopathy [153].

More than ¾ of affected individuals present with seizures in the neonatal period. The phenotype is characteristic with a severe progressive encephalopathy developing a few hours to days after birth, associated with multifocal, erratic, myoclonic jerks and clonic seizures. Hiccup and gastrointestinal symptoms, including vomiting are often present. Particularly, hiccups may be felt in utero. Neonates tend to progress to apnea requiring ventilator support [154,155]. EEG can be normal in the first hours after birth but rapidly evolves into a burst-suppression pattern consisting of periods of high amplitude activity lasting 1 to 3 seconds that arise periodically from a markedly depressed background with lack of organization and physiological features. The bursts are mostly asynchronous over both hemispheres and comprise irregular sharp waves and spikes. This pattern tends to evolve into hypsarrythmia by the end of the first month [116]. About 85% of neonates present a severe form characterized by profound developmental delay, tetraparesis, and intractable seizures. Fifteen percent of neonates have some residual enzyme activity resulting in an attenuated phenotype. The attenuated phenotype is characterized by variable developmental progress, hyperactivity, chorea, intermittent ataxia, and behavioral problems [155].

Brain MRI sometimes reveals anomalies including corpus callosum hypoplasia or hydrocephalus, always predicting a poor outcome [154,155]. On diffusion weighted imaging (DWI) symmetrical bright signal intensity in the posterior limb

of the internal capsule, lateral thalamus, dorsal pons, midbrain, cerebellar white matter, and globus pallidus is suggestive of the diagnosis [156]. Magnetic resonance spectroscopy (MRS) has been used for non-invasive measurement of brain glycine levels in patients with glycine encephalopathy [157,158]. The use of MRS improves the early diagnosis of patients with glycine encephalopathy.

Cerebrospinal fluid (CSF) levels of glycine are very elevated. Significant increases in serum glycine levels are often also seen, although these levels may be within normal range in affected patients, mandating the need for CSF examination. A CSF/serum glycine ratio of >0.08 (with both samples taken simultaneously) is considered diagnostic for the disorder. Diagnostic confirmation is obtained by DNA analysis.

The aim of current therapy is to decrease glycine concentrations and block the effect of glycine at the neurotransmitter receptors. Glycine plasma concentrations can be reduced by benzoate and low protein or ketogenic diet. Glycine, benzoate and carnitine should be monitored regularly during treatment. NMDA receptor antagonists including dextromethorphan might be helpful in attenuated forms. Combined anticonvulsant treatment is necessary, but often ineffective in children with severe glycine encephalopathy. Valproate is contraindicated as it further decreases enzyme activity [155,159,160]. Accurate genotype-phenotype correlation allows genetic testing to play an increasing role in the neonatal period when decisions regarding medical intervention are made. An attenuated phenotype of nonketotic hyperglycinemia can be predicted when a mutation with residual enzyme activity is identified. A mutation with residual activity is necessary but not sufficient for an attenuated phenotype [153].

Even when optimal treatment is promptly initiated, the prognosis depends on residual enzyme activity. In patients with no residual enzyme activity (severe phenotype), the prognosis is invariably poor leading to redirection of care in many cases. In patients with an attenuated phenotype, early initiation of treatment has been associated with better developmental outcome [161,162].

SULFITE OXIDASE DEFICIENCY

Sulfite oxidase deficiency is an autosomal recessive disorder due to mutations in *SUOX* gene. Neonates present with intractable seizures and encephalopathy, arising in the first few hours to days of life, sometimes mimicking hypoxic-ischemic encephalopathy. They exhibit feeding difficulties and tone abnormalities, including opisthotonus, followed by acquired microcephaly, spastic quadriplegia with pyramidal signs, abnormal movements, and profound intellectual disability [163,164]. The MRI shows a loss of grey-white matter differentiation and cytotoxic

edema in the cerebral cortex, subcortical white matter, and basal ganglia. These findings contrast with the ones found in hypoxic-ischemic encephalopathy where the cerebellum, brain stem, and deep gray matter structures are usually spared. Magnetic resonance spectroscopy may show reduced N-acetylaspartate/total creatine ratio and abnormal elevation of lactate, glutamine, and glutamate. Brain injury evolves into multicystic white matter lesions and severe brain atrophy. The outcome is invariably poor with severe developmental disabilities and early death. No specific treatment exists [164].

MOLYBDENUM COFACTOR DEFICIENCY

Similar to sulfite oxidase deficiency, molybdenum cofactor deficiency is a rare autosomal recessive condition that presents within the first hours to days of life with abrupt onset of encephalopathy, cerebral edema, and refractory seizures [165]. Early MRI may show acute changes consisting of global cerebral edema evidenced by hyperintense signal on diffusion-weighted imaging (DWI), and hypointense signal on calculated apparent diffusion coefficient maps. These maps tend to "pseudo-normalize" by 7-10 days after birth. The MRI then evolves into a pattern of cystic encephalomalacia with cortical and subcortical atrophy. Interestingly, there have been reports of neonates with cystic encephalomalacia, including basal ganglia and thalamic injury, present already in the very first days of life, as well as fetuses with multiple subcortical cavities, ventriculomegaly, dysgenesis of the corpus callosum, and hypoplastic cerebellum with an enlarged cisterna magna, suggesting that brain injury may already start in utero [166].

Two-thirds of the cases are due to mutations in the *MOCS1* gene, which catalyzes the first step in the synthesis of molybdenum cofactor. Patients with molybdenum cofactor deficiency generally have severe neurodevelopmental outcomes and die at a median age of 3 years [167]. Schwahn and colleagues reported on three children treated before the onset of seizures and encephalopathy, with daily infusions of cyclic pyranopterin, which resulted in nearly normal development [168]. However, in most cases, treatment is started after the occurrence of brain injury and does not improve neurodevelopmental outcomes, despite correction of the metabolic abnormalities, suggesting a very narrow temporal window for effective intervention. Heightened clinical suspicion and early evaluation for decreased serum and urine uric acid and elevated sulfite, S-sulfocysteine, xanthine, and hypoxanthine are critical for timely diagnosis and treatment.

SLC13A5-ASSOCIATED EPILEPTIC ENCEPHALOPATHY

Mutations in *SLC13A5* gene have recently been described to cause an autosomal recessive epileptic encephalopathy. *SLC13A5* encodes a high affinity sodium-

dependent citrate transporter, which is expressed in the brain and liver. *SLC13A5* mutations result in loss of citrate transport leading to brain energy failure, an imbalance in glutamate and GABA production, and reduced inhibition of the excitatory NMDA receptor [169]. Seizures often start in the first days after birth, with progression into refractory epilepsy, status epilepticus, and early death. In survivors, developmental delay and persistence of epilepsy and status epilepticus is common [140,169]. Interestingly, interictal EEG can be normal initially or show only some discontinuity. Brain MRIs exhibit an early characteristic pattern, with punctate white matter lesions, which are no longer visible at 6 months of age, but lead to gliotic scarring visible on MRI at 18 months [140]. Genetic analysis confirms the diagnosis.

3.B. CHANNELOPATHIES

KCNQ2-ASSOCIATED NEONATAL EPILEPSY

The *KCNQ2* gene encodes the voltage-gated potassium channel K_V *7.2.* This potassium channel underlies the muscarine-regulated M-current, a widely distributed slow activating and non-activating current, which plays a dominant role in modulating neuronal excitability by causing spike-frequency adaptation and setting the subthreshold membrane potential [170]. Mutations in *KCNQ2* and *KCNQ3* genes, were identified as a cause of BFNE in 1998. These were the first epilepsy gene to be discovered [171,172]. More than 10 years later, *KCNQ2* was surprisingly also linked to a severe epilepsy syndrome named *KCNQ2* encephalopathy [130]. The fact that mutations in the same gene can give rise either to a self-limiting epilepsy or a severe epileptic/developmental encephalopathy demonstrates the importance of *KCNQ2* in brain development and suggests that the resulting potassium currents may be differently affected in the two diseases. Interestingly, children with KCNQ2 pathogenic variants at the same position were found to have both a benign (R213W) and a severe (R213Q) form suggesting a differential impact on the protein based on the amino acid change. A functional study comparing the impact of these mutations showed that more severe functional deficits are associated with more severe epileptic phenotypes [173]. All four gates of the tetramer must work in tandem for the KCNQ channel to properly allow ion flow. If one of the subunits has a mutation leading to electromechanical uncoupling or pore-blocking, the tetramer becomes non-conducting, despite 3 normal wild-type subunits. This dominant-negative effect is exhibited by many of the mutations leading to *KCNQ2* encephalopathy with a pronounced loss of the M-current in the severe phenotypes [174].

The severe phenotype seen in *KCNQ2* encephalopathy may be further explained by the separation of the mutated potassium channels from their usual axonal partner, the voltage-gated sodium channels. Ankyrin-G is a sub-membranous framework that binds potassium and sodium channels together in the axon initial segment. The failure of appropriate clustering of channels in the membrane can lead to abnormal excitability [170,175]. This theory provides further grounds for the development of pharmacologic interventions that could reconnect or bind the channels and prevent the excessive or sustained firing of action potentials seen with seizures.

Finally, recent work has revealed a previously unexplored level of complexity in pathogenic mechanisms underlying *KCNQ2* encephalopathy. Indeed, some of the mutations stabilized the activated state of the channel, thereby producing gain-of-function effects [176]. However, careful analysis of the early phenotype of these patients showed that instead of the typical neonatal epileptic encephalopathy, these neonates present with encephalopathy, a burst-suppression pattern on EEG, and non-epileptic myoclonus, often misdiagnosed as seizure [177]. This clinical presentation is distinct from the phenotype associated with loss-of-function variants, supporting the value of in vitro functional screening.

BENIGN FAMILIAL NEONATAL EPILEPSY (BFNE)

BFNE is associated, in over 80% of cases, with mutations or deletions in the potassium channel genes KCNQ2 or less often KCNQ3 [9]. BFNE mostly occurs as an autosomal dominant disorder with incomplete penetrance, although cases of de novo germline mutations with a mild effect or post-zygotic mosaicism for a more severe variant in KCNQ2 gene have been described [137]. Screening for mosaicism and accurate variant classification in individuals with apparent de novo KCNQ2 mutations is crucial and has important implications for genetic counseling.

In a recent multicenter study, BFNE was responsible for about 3% of all neonatal seizures [4]. Seizures occur within the first days after birth and have a pathognomonic semiology, characterized by asymmetric tonic posturing, sometimes evolving to unilateral or asynchronous bilateral clonic movements, shifting laterality, often accompanied by apnea and oxygen desaturation. Seizures last 1 to 2 minutes and can occur as frequently as 30 times per day, sometimes leading to status epilepticus. On EEG, the interictal background activity is continuous and well organized without focal slowing or attenuation to suggest an underlying lesion. Bilateral independent epileptiform abnormalities, mainly over the central regions, can be present [9]. A distinctive ictal pattern has been described on aEEG consisting of a sudden rise of the lower and upper margin,

followed by a marked depression of the aEEG amplitude [10]. Neurodevelopmental outcome is generally good, although it has been reported that up to 25% of patients develop epilepsy later in life. The recurrence risk seems to correlate with seizure burden in the neonatal period [178]. In the context of a positive family history, recognition of the typical electro-clinical phenotype should allow for rapid suspicion of BFNE, without the need for extensive ancillary testing [9,10]. In a recent multicenter study of 19 patients, low dose oral carbamazepine (10mg/kg/day) or oxcarbazepine has been reported to be safe and rapidly effective, even in the context of status epilepticus, and was associated with shortened hospital stay [9]. Carbamazepine is a sodium-channel blocker seldom used in the NICU [179–181]. It has been used in children and adults for over 30 years to treat focal seizures with an excellent therapeutic and side effect profile. Interestingly, it is effective in focal epilepsy involving the supplementary sensorimotor area that resembles seizures presented by infants with KCNQ2-related epilepsies [182].

KCNQ2 ENCEPHALOPATHY

On the other end of the spectrum, *de novo KCNQ2* variants leading to loss-of-function are responsible for over 10% of severe early-onset epileptic encephalopathies [131]. Nearly 90% of mutations leading to *KCNQ2* encephalopathy occur in 4 high-risk zones. This strongly supports the fact that dominant-negative suppression is the primary factor underlying severe phenotypes in most cases of *KCNQ2* encephalopathy [174].

KCNQ2 encephalopathy is characterized by onset, within the first week after birth, of focal tonic seizures, similar in semiology to the ones seen in BFNE [8], associated with encephalopathy (hypotonia, paucity of spontaneous movements, no visual fixation, and altered reactivity). The neonatal EEG background demonstrates multifocal epileptiform abnormalities with random attenuation or burst suppression. The ictal EEG shows the onset of low voltage fast activity over a single hemisphere followed by focal spike and wave complexes. While seizures are quite short in duration, the post-ictal phase is typically characterized by marked and prolonged diffuse voltage attenuation. Seizure frequency is high with 10 or more seizures per day or even per hour. Seizures tend to remit in early childhood. However, most children have severe intellectual disabilities exemplifying the concept of DEE [131]. This highlights the need for therapeutic approaches that address the developmental outcome in addition to the seizures, by targeting the underlying pathophysiology directly. As in BFNE, sodium channel blockers such as carbamazepine and phenytoin have been shown to be particularly effective [8,183] and are now considered an established precision medicine treatment for *KCNQ2*-related epilepsies [184].

Gain-of-function variants (R201H and R201C) have also been reported to cause *KCNQ2* encephalopathy although they are associated with a different phenotype than the loss-of-function variants. In this case, while epileptic seizures are not seen in the neonatal period, the clinical presentation is characterized by encephalopathy from birth, irregular breathing patterns, and prominent startle-like non-epileptic myoclonus, which can be triggered by sound or touch. The EEG in the neonatal period shows a burst-suppression pattern. Some neonates subsequently develop infantile spasms, and all have profound developmental delay. Interestingly, one patient had a later onset, and sequencing indicated that a low abundance (~20%) R201C variant had arisen by postzygotic mosaicism [177].

KCNT1-ASSOCIATED EPILEPSY

KCNT1 encodes a weakly voltage-dependent sodium-gated potassium channel subunit also known as SLACK (Sequence Like a Calcium Activated K+ channel). It is widely expressed in the nervous system and represents the largest known potassium channel subunit. Its activity contributes to the slow hyperpolarization that follows repetitive firing of action potential [185]. Functional studies show that *KCNT1* mutations causing EIMFS are associated with a gain-of-function phenotype in vitro, leading to increase current amplitude in the sodium-activated potassium channel [111,186]. However, no correlation has been found between the magnitude of the impact on the protein and the phenotype.

The first disease-causing mutation in *KCNT1* was described in 2012 associated with epilepsy of Infancy with Migrating Focal Seizure (EIMFS) [111]. EIMFS had been first described in 1995 by Coppola and Dulac, as a disease characterized by nearly continuous multi-focal partial seizures arising independently and sequentially from both hemispheres, progression through a period of intractable seizures, subsequent neurologic deterioration or arrest with complete loss of both cognitive and motor abilities, and in most children, progressive microcephaly [187]. Although this disorder was initially described in infants, many neonatal cases have subsequently been reported. The first seizures occur before 6 months of age (1 day -6 months; mean 25 days) in previously normal infants. During the first phase of the disease, focal seizures with a unilateral motor onset may be sporadic and alternating from one side of the body to the other with lateral deviation of head and eyes, limb myoclonic jerks and increase tone in one or both limbs. In half of the patients, seizures are associated autonomic symptoms, such as apnea and desaturation, and are sometimes misdiagnosed as gastro-esophageal reflux. Electrical seizures without clinical correlates have also been reported. EEG background may initially be normal or show diffuse slowing but soon becomes abnormal with lack of organization, and frequent seizures with shifting laterality between recordings. Electrographic seizures are monomorphic

in each patient consisting of focal rhythmic alpha or theta beginning in one region and progressively involving the adjacent areas. The ictal onset locations vary. During the second phase, which may already start in the first month of life, seizures become very frequent, occurring in clusters of 5 to 30 seizures during drowsiness and awakening several times a day or being almost continuous for days or weeks. During clusters, between seizures, infants are often hypotonic and somnolent. They may partially recover between clusters. However, as soon as the next cluster occurs, infants regress. With time, seizures tend to generalize more frequently. EEG shows multifocal paroxysmal abnormalities affecting alternatively both hemispheres with migrating features characterized by multiple independent prolonged seizures evolving simultaneously from different regions of the brain. Seizures tend to be highly refractory to both conventional ASMs and corticosteroids. However, some response has been reported with potassium bromide [188,189] and levetiracetam [190,191]. About 25% of patients die within a year from onset. After 1 to 5 years, seizures may become less frequent, but there is severe developmental disability [187,192].

While *KCNT1* mutation could account for the increased excitability and seizure phenotype, the severity of the developmental delay in children with EIMFS suggests an independent developmental role for this gene. In addition to regulating ion flux, a number of channels have non-conducting functions that regulate biochemical activities independent of ion flux [193]. This is likely to be the case for the KCNT1 channel, which, in its C-terminal domain, interacts with a protein network including fragile-X mental retardation protein. *KCNT1* mutations may alter the conformation of the C-terminal region of the protein, impairing not only the gating of the channel but also its ability to interact with developmentally relevant proteins such as FMRP, and accounting for the severe developmental delay [111].

KCNT1 mutations have also been associated with other types of epilepsies occurring later in life such as autosomal dominant nocturnal frontal lobe epilepsy. In some families, the same mutation can lead to both EIMFS and autosomal dominant nocturnal frontal lobe epilepsy illustrating the phenotypic heterogeneity phenomenon.

Quinidine, a class I antiarrhythmic drug, was found to reverse *in vitro* the increased conductance of *KCNT1*. Trials of quinidine in 17 patients with *KCNT1* mutations have been reported with mixed results [114,186,194–198]. None of the 10 patients aged more than 4 years at the time of treatment responded to quinidine. Of these, 8 had seizure onset after 1 year of life. In the remaining 2 who did not respond to quinidine, while seizure onset was in the neonatal period, no migrating focal seizures are described. In contrast, all 4 patients treated before 4

years of age responded to quinidine (3 had a diagnosis of EIFMS and one had infantile spasms). For 3 additional patients with EIFMS treated with quinidine, the age at time of treatment is unknown. One showed seizure reduction, 1 had no effect and in the third one, treatment was discontinued due to severe vasculopathy [186]. Hence, genotype alone may not be sufficient to investigate this targeted treatment efficacy and accurate phenotyping, as well as appropriate timing, might be crucial elements. Additional studies are needed to better assess efficacy, optimal age at the time of treatment, dose, route of administration, and pharmacokinetics. However, the use of quinidine in EIMFS represents the promise of what precision medicine can offer for intractable epilepsies.

SCN2A-ASSOCIATED NEONATAL EPILEPSY

SCN2A pathogenic variants are also responsible for a channelopathy associated with a wide phenotypic spectrum including neonatal forms and later-onset disease including epilepsy, developmental delay, and autistic spectrum disorders.

SCN2A encodes Nav1.2, a major voltage-gated sodium channel in the central nervous system early in development. Nav1.2 is supplanted over time, to some extent, by Nav1.6 (SCN8A), which may account for the limited temporal expression of *SCN2A*-associated epilepsy [199]. A recent large study reported on the genotype-phenotype presentation of 201 patients with *SCN2A* mutations half of whom had seizure onset before 3 months of life. Interestingly, patients with early seizure onset (<3mo) presented with missense mutations resulting in a gain of function of the sodium channel, whereas patients with a later onset often had loss of function mutations. The severity of the gain of function correlated with the severity of the clinical phenotype [200].

One of the typical presentations of *SCN2A* mutations is Benign Familial Neonatal-Infantile epilepsy (BFNIE). In patients with BFNIE, seizures usually start between 2 days and 3 to 6 months of life. Although half of the patients present in the neonatal period, there is heterogeneity within the same family, and different members with the same mutation may have a different age at onset (phenotype heterogeneity). Seizures tend to occur in clusters and are predominantly focal tonic with alternating laterality from seizure to seizure. The interictal EEG is normal or shows occasional focal spikes. Seizures abate within the first two years of life with low recurrence risk and good neuro-developmental outcomes although cases of episodic ataxia later in life have been reported with two specific mutations (A263V and R1882G) [200,201].

On the other end of the spectrum, *de novo* missense variants in *SCN2A* can be associated with refractory epilepsy presenting either in the neonatal period (neonatal-onset) or in the infantile period (late-onset). The predominant seizure

types in the neonatal form are focal seizures with a tonic component and apnea. The initial EEG is abnormal with a burst suppression pattern or multi-focal spikes. In the late-onset form, patients typically present with epileptic spasms. Patients have severe intellectual disabilities, axial hypotonia, and microcephaly, sometimes accompanied by a movement disorder [200].

Analogous to the situation with *KCNQ2*, potential benefit of sodium channel blockers, such as carbamazepine and phenytoin, has been suggested [202]. It seems that sodium channel blockers, by counteracting the increased conductance through the mutant sodium channels, decrease the effect of the gain of function mutation. Interestingly in patients with loss of function mutation, usually presenting later in life, sodium channel blockers have been shown to worsen the disease [200].

3.C. DISORDERS OF THE CELL SIGNALING

CDKL5 ENCEPHALOPATHY

The *CDKL5* (cyclin-dependent kinase-like 5) gene is located on the short arm of chromosome X (Xp22) and plays a crucial role in brain development. Dominantly acting loss-of-function variants are responsible for this early onset epilepsy associated with encephalopathy affecting mainly girls. Pathogenic CDKL5 variants are found across the coding regions of the gene but missense variants clearly cluster in the N-terminal catalytic domain suggesting that the kinase function of CDKL5 is particularly important for brain function. Genotype-phenotype correlation studies have found that mutations in the N-terminal kinase domain are often associated with more severe clinical symptoms than mutations in the C terminus [203,204]. However, some patients harboring the same mutation have shown difference in the severity of the epileptic encephalopathy. This could be due to X-chromosome random inactivation, resulting in patients carrying the same genetic mutation having different mosaic expression patterns of *CDKL5*, thus resulting in a spectrum of phenotypes [139,204].

In *CDKL5* encephalopathy, seizure onset is around 3-6 weeks of life. At onset, seizures are brief but frequent and often intractable. During this early stage, the interictal EEG may remain normal. Some of these infants experience successful seizure control after several weeks to months. Subsequently, the EEG background deteriorates, and the developmental delay, including hypotonia, lack of head control, and poor eye contact becomes evident. Seizures often display a distinctive “hypermotor-tonic-spasms” sequence [120,205]. Interestingly, epileptic spasms can be present with or without hypsarrhythmia. Later on, there is evolution into multifocal and myoclonic epilepsy [120,139,206]. Marked motor

and cognitive impairment associated with feeding and sleep difficulties are typical [207]. In addition, a significant proportion of patients show a deceleration of head growth and some form of hand stereotypes somehow like the one seen in girls with Rett syndrome. Some boys with very severe early onset phenotype or mosaicism for the pathogenic variant have been reported [208,209].

The mechanism by which a mutation in the *CDKL5* gene can lead to such a severe and early-onset epileptic encephalopathy is not yet fully understood. One of the functions of the CDKL5 protein is to phosphorylate other proteins, and one of its major targets is *MECP2*, known to be involved in neuronal function and synaptic maintenance. *MECP2* mutations are associated with Rett syndrome, a disease that shares some similarities with *CDKL5* encephalopathy. Two *CDKL5* knockout mouse model have been described [210,211]. Behavioral analysis reveals that the knockout model reproduces key features of the human disorder, including limb clasping, hypo-activity, and abnormal eye tracking. Investigations of these mice models have uncovered potential substrates for the *CDKL5*-associated encephalopathy, including reduced dendritic arborization of cortical neurons, decreased visual evoked responses, and alterations in Akt/rpS6 signaling pathways. Particularly interesting is the dissociation of behavioral phenotypes resulting from a conditional knockout of *CDKL5* in glutamatergic cortical neurons and GABAergic forebrain neurons respectively. These findings suggest that the behavioral phenotype (hypomotricity) observed in *CDKL5* encephalopathy derives from the localized absence of CDKL5 in the GABAergic forebrain neurons, and that the limb control and eye tracking phenotypes depend on glutamatergic neurons. However, these mice models lack spontaneous seizures or epileptiform activity, which are key features of *CDKL5* encephalopathy.

BRAT1-ASSOCIATED LETHAL NEONATAL RIGIDITY AND MULTIFOCAL SEIZURES

Mutations in *BRAT1* gene have been associated with Lethal Neonatal Rigidity and Multifocal Seizures (LNRMFS) syndrome. We will describe this ultra-rare disorder in this chapter, PART C (page 85). Little is known about the neonatal electro-clinical phenotype of *BRAT1* related conditions. Neonates present with diffuse hypertonia, microcephaly, multifocal myoclonic jerk with and without EEG correlates, and multifocal refractory seizures leading to early death often in a context of refractory apnea and bradycardia [212–216]. *BRAT1* mutations have also been associated with a milder presentation characterized by either later-onset epilepsy and survival past infancy or intellectual disability, ataxia/dyspraxia, and cerebellar atrophy with or without epilepsy [217–219].

Intra-familial phenotypic variability has been reported with siblings presenting with forms of variable severity [220]. Early genetic testing helps to prevent unnecessary procedures and futile treatments even if it remains unreliable to predict clinical outcomes from genomic data.

Genetic counseling is critical as recurrence risk in parents from a first affected child is 25%, as for any autosomal recessive condition. Families with 2 or more siblings affected by LNRMFS have been described.

GNAO1-ASSOCIATED ENCEPHALOPATHY

Around 30 patients have been reported with *GNAO1*-associated encephalopathy, 10 of them presenting with neonatal-onset epileptic encephalopathy. In these neonates, seizures tend to be focal. EEG background is abnormal with either a burst-suppression pattern or multifocal sharp waves. The main neuroimaging features are cerebral atrophy, delayed myelination, and a thin corpus callosum [47].

$G\alpha_o$, encoded by *GNAO1*, is extremely abundant in brain tissue, where it can constitute up to approximately 0.5% of membrane protein, suggesting important roles in brain function. Mice lacking $G\alpha_o$ show multiple neurological abnormalities, including generalized tremor, occasional seizures, severe motor-control impairment, hyperalgesia, and behavioral abnormalities with early postnatal lethality [221].

3.D. DISORDER OF THE SYNAPTIC TRANSMISSION

STXBP1 ENCEPHALOPATHY

De novo mutations in *STXBP1* (syntaxin-binding protein 1) gene are one of the major causes of neonatal-onset developmental encephalopathy associated with epilepsy most often presenting as OS [138].

The *STXBP1* gene, also known as MUNC18-1, is predominantly expressed in the brain. It encodes for a membrane trafficking protein, which plays an important role in synaptic vesicle docking and fusion, a necessary mechanism for neurotransmitter secretion. Through interaction with both vesicle-associated (synaptobrevin 2) and target-associated (syntaxin-1) soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) proteins, STXBP1 modulates the presynaptic vesicular fusion reaction. Syntaxin-1 include both an N-terminal peptide (N-peptide) and a large conserved Habc-domain that bind to STXBP1. While N-peptide is essential for vesicle fusion as such, the H_{abc}-domain regulates the fusion, in part by forming the closed syntaxin-1

conformation. Hence the complex structure of STXBP1 is required for its multifaceted role in different types of synaptic vesicle fusion [222].

An *STXBP1* knockout mouse model showed that total disruption of STXBP1 leads to a complete loss of neurotransmitter secretion from synaptic vesicles. *STXBP1* knockout mice further showed neurodegeneration after an initially normal brain assembly, indicating that neuro-transmitter secretion, and thus functional STXBP1, is important for the maintenance of neuronal synapses. Reduced *STXBP1* expression was further shown to increase synaptic depression at both GABAergic and glutamatergic synapses with a greater impact on GABAergic interneurons. This might result in a net hyperexcitability and epileptic activity in case of *STXBP1* haploinsufficiency [138,223]. These studies shed light on the pathophysiology of OS, which, in the setting of normal brain architecture, could be related to altered neurotransmission.

STXBP1 mutations have initially been described in patients diagnosed with OS [224]. Later, *STXBP1* was also associated with unclassified early-onset epileptic encephalopathy, and a variety of phenotypes including West syndrome, Dravet syndrome, non-syndromic epilepsy and intellectual disability, and autism [138].

Ninety-five percent of patients with *STXBP1* pathogenic variants have epilepsy. In patients with *STXBP1* encephalopathy, seizures tend to occur early in life, often in the neonatal period (median age at onset: 6 weeks). Neonates tend to present with epileptic spasms, often associated with refractory focal seizures. EEG at onset is markedly abnormal, usually showing a burst suppression pattern, sometimes multi-focal abnormalities. Half of the patients evolve to West syndrome. They have moderate to severe cognitive impairment and normal head circumference [138,225]. Epilepsy may settle down relatively early in the child's history, but the developmental consequences often remain profound. Affected infants also tend to develop an ataxic gait and paroxysmal non-epileptic movements later in childhood [225,226].

Recently 3 cases of focal cortical dysplasia (FCD) associated with *STXBP1* mutation raised the possibility of a causal relationship between neuronal expression of *STXBP1* mutations and FCD. One patient with *STXBP1* mutation, early infantile epileptic encephalopathy, developmental delay and autism spectrum disorder achieved complete seizure remission following resection of dysplastic brain tissue. Examination of excised brain tissue identified mosaicism for *STXBP1*, providing evidence for a somatic mechanism [227]. This finding, although very preliminary, also highlight the links between genetic and structural epilepsies.

4. TREATMENT OF NEONATAL-ONSET EPILEPSY: FROM ONE SIZE FITS ALL TO PRECISION MEDICINE

For many years, seizures in neonates have been lumped all together, considered as a single entity, and treated using protocols that did not account for etiology as a factor. Advances in our understanding of the genetic basis of neonatal epilepsies have opened the way for precision medicine approaches rather than a "one size fits all" strategy. For example, phenobarbital has been shown to be the most effective anti-seizure medication for seizures in neonates with HIE. However, *KCNQ2*- and *KCNQ3*-related epilepsies do not respond to phenobarbital and have been demonstrated to selectively respond with seizure-freedom to sodium channel blocking ASMs including carbamazepine and phenytoin.

The first step in any cure is the diagnosis, and any effective cure will benefit from an early diagnosis. Currently, many neonates with genetic epilepsies remain undiagnosed until later in life without an opportunity for early intervention. Precision medicine in the NICU offers a unique opportunity if we embrace therapeutic approaches stratified by etiology.

While the first challenge is early recognition, another challenge will be for flexibility in the use of novel treatments as they are identified. Novel therapeutic approaches for neonatal epilepsies have been either the result of attentive clinical observation (carbamazepine in *KCNQ2*-related epilepsies), or rationally directed therapy with *in vitro* validation (quinidine in EIMFS associated with *KCNT1* mutations). Therapies for treating rare diseases need their efficacy and safety evaluated in prospective studies to confirm the initial observations. Due to the small number of potential participants, trials are not feasible without large international collaboration to recruit neonates into trials of targeted novel therapeutics when they are most likely to have a beneficial impact. Enhancing the collaboration between neonatologists, epileptologists, and neonatal neurologists in different parts of the world is the key to overcoming those challenges.

PART B: DISTINCTION BETWEEN ACUTE PROVOKED SEIZURES AND NEONATAL-ONSET EPILEPSIES

Seizures resulting from neonatal-onset epilepsies must be addressed differently from acute provoked seizures [228,229]. While fetal and postnatal MRI can easily detect structural brain malformations, the early diagnosis of genetic MRI-negative epilepsies is challenging. Genetic neonatal epilepsies are frequently associated with significant cognitive and behavioral morbidity [230,231]. In patients with genetic epilepsies, seizures may respond particularly well to select agents, contrasting with the nonspecific approach traditionally used in the neonatal intensive care unit (ICU) [232]. Early effective treatment could mitigate the effects of seizures on the developing brain, but this entails recognizing seizures and diagnosing epilepsy when it presents in the nursery.

Identifying seizures and interpreting seizure type is challenging in adults [233] and children [234], and even more so in neonates [235,236]. Nevertheless, a recent systematic review of the literature [237] suggested that seizure semiology in neonates may have diagnostic value with respect to etiology and thus implications for management.

The high yield of targeted exome analysis in neonates with genetic epilepsies [6,238] supports early genetic testing in infants with certain seizure phenotypes [239], thus providing prognostic and genetic information for family planning and patient care and averting further unnecessary diagnostic testing. As most neonates with seizures have acute provoked seizures, those with genetic epilepsies run the risk of being treated as such with medications that are ineffective, missing the window of opportunity for early targeted intervention. This can be prevented if providers have the clinical skillset to identify neonates with genetic epilepsy as different. Even when genetic testing returns a putative finding, confirmation of the epilepsy diagnosis with accurate phenotypic data is essential [36].

We hypothesized that neonates with genetic epilepsies present differently from neonates with acute provoked seizures. We analyzed the video-EEGs (vEEG) of neonates with seizures recorded at seizure onset, to describe the early semiology of seizure in neonates with genetic epilepsies and compare it with the semiology of neonates with acute provoked seizures.

1. MATERIAL AND METHODS

This study was approved by the Committee on Human Research at UCSF and the Ethical Committee at Saint-Luc University Hospital, UCLouvain, and a waiver for informed consent was granted.

Population: Neonates with genetic epilepsies were identified by a systematic review of all neonates ≤ 44 weeks' corrected gestation who received EEG monitoring and had a diagnosis of seizure in two level IV neonatal ICUs (Benioff Children Hospital, San Francisco, USA, and Cliniques universitaires Saint-Luc, Brussels, Belgium. Neonates for whom vEEG recordings were available for review were included. A subset of patients has been previously published [240,6].

Genetic epilepsy was defined as a condition resulting directly from a known 'pathogenic' or 'likely pathogenic' genetic variant in which seizures are a core symptom of the disorder [46,53]. Genetic testing was performed on a clinical basis. Counseling and consent for genetic testing were conducted by the treating clinicians and the clinical result reports were evaluated by the investigators. Variants were identified on either epilepsy targeted panels, or whole exome sequencing. Single nucleotide polymorphism arrays or comprehensive genomic hybridization arrays were performed to search for copy number variation. The variants detected were queried against the Exome Variant Server database (evs.gs.washington.edu) to determine their prevalence in large control populations. Polyphen-2-software (genetics.bwh.harvard.edu/pph2) was used to determine in silico likelihood of pathogenicity. Clinical significance of variants and inheritance patterns were queried using the ClinVar (ncbi.nlm.nih.gov/clinvar) and OMIM (ncbi.nlm.nih.gov/omim) databases [46].

Subsequently, this group of neonates with genetic epilepsies was compared to consecutive neonates with acute provoked seizures due to either HIE or stroke, the most common etiologies of seizures in this age group, in whom vEEG was recorded between January 2017 and December 2019 in UCSF, who were discharged seizure-free and without a diagnosis of epilepsy. We choose 40 neonates born consecutively with to avoid any selection bias in our control cohort. Neonates who were paralyzed and neonates for whom video of seizures was not available for review were excluded. We choose to enroll 2 patients with acute provoked seizures for each patient with genetic epilepsy to increase our statistical power to show a difference in frequency of tonic seizures between groups. Based on effect size calculation, assuming 10% of neonates with acute provoked seizure would have tonic seizures, and that we would include 20 neonates with genetic epilepsies and 40 (2:1 ratio) with acute provoked seizures we would have 90% power to detect a difference between the rate of tonic seizures among neonates

with acute provoked seizures and the ones genetic epilepsies >= 50%, with a two-sided alpha error set at 0.05. We believe this difference would be both clinically meaningful and plausible.

Video EEG analysis: vEEG recording was promptly initiated in neonates with encephalopathy and/or clinical events suspicious for seizures and continued until at least 24 hours after the last seizure [241]. In addition, aEEG was displayed at the bedside for real time evaluation by the neonatology team. Neonates with HIE were monitored upon admission during the whole duration of therapeutic hypothermia and through rewarming. vEEG recordings were analyzed by an experienced neonatal neurophysiologist (MRC) and a neonatologist with expertise in neonatal EEG (MCC) for the purpose of the study. Clinical seizures without EEG correlate were not considered.

Seizure semiology was classified according to the new ILAE classification [49] as (1) clonic (2) tonic (3) myoclonic and (4) electrographic only. The new ILAE classification also includes as a new category sequential seizure. For this work, in the interest of clinical applicability, seizures with an initial well-defined tonic phase followed by clonic or myoclonic jerks were classified as tonic. Tonic seizures were further classified as either tonic only or tonic sequential (initial tonic phase followed by either clonic or myoclonic jerks).

Data analysis and statistical methods: Demographic data were collected from the electronic health record and managed using REDcap electronic data capture tools hosted at UCSF Benioff Children's Hospital [242]. Univariate statistical analyses were performed using the t-test or Wilcoxon Rank Sum (Mann-Whitney *U*) test for continuous variables and the Fisher's exact test for categorical variables.

2. RESULTS

Twenty patients with genetic epilepsies developed seizures at less than 44 weeks of corrected gestational age, had vEEG recording available for review, and were analyzed. Their presentation and genetic finding are described in the Annex 1 and 2. We compared them with 40 term neonates with acute provoked seizures: 14 (35%) due to stroke and 26 (65%) due to HIE.

The demographics and seizures characteristics of neonates included in this study are presented in Table 7.

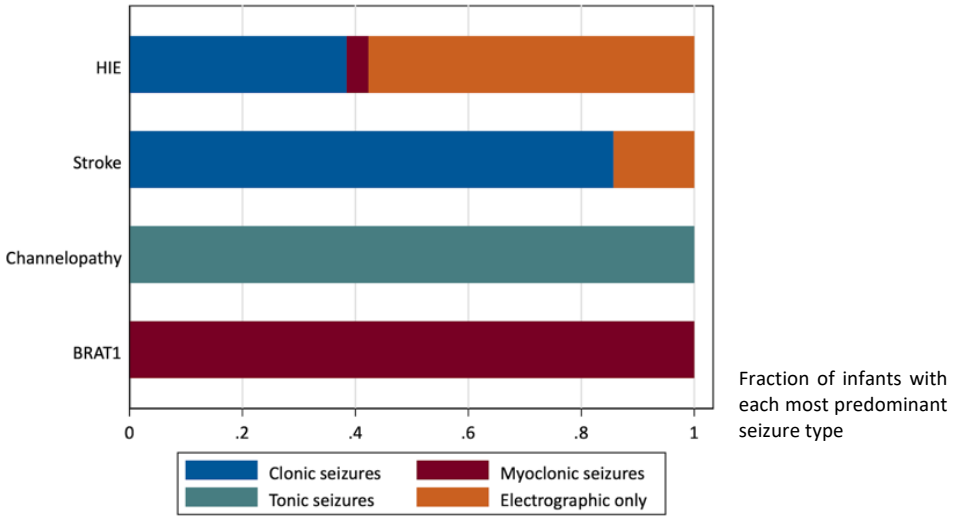
Table 7: Characteristics of neonates with genetic epilepsies and APS

	Genetic epilepsies (n=20)	Acute provoked seizures (n=40)	p-value
DEMOGRAPHICS			
Male	10 (50%)	15 (38%)	0.35
Gestational age (weeks)	38.5 (38-40)	40 (39-41)	<0.001
Birthweight (Kg)	3.3 (2.7-3.6)	3.2 (3.0-3.6)	0.60
Apgar score 5min	9	5 (2-7)	<0.001
Family history of neonatal seizure	9 (45%)	1 (3.3%)	<0.001
SEIZURES			
Age at seizure onset (hours)	60 (26-96)	15 (10-22)	<0.001
Treated with ≥ 2 ASM	16 (80%)	13 (32%)	0.001
All seizures were clinical	11 (55%)	4 (10%)	<0.001
All seizures were subclinical	0 (0%)	17 (42%)	<0.001
1 or more clonic seizure	3 (15%)	22 (55%)	0.005
1 or more tonic seizure	18 (90%)	0 (0%)	<0.001
1 or more myoclonic seizure	2 (10%)	1 (3%)	0.44

Video-EEGs of seizures recorded before the administration of any anti-seizure medication (ASM) were available in eight of 20 (40%) neonates with genetic epilepsies and 35 of 40 (87%) neonates with acute provoked seizures.

The seizure type in the 40 neonates with acute provoked seizures, was mostly clonic or electrographic only, as 22/40 infants (55%) had at least one clonic seizure. Clonic seizures were more common in neonates with stroke (12 of 14, 86%) than in HIE (10 of 26, 38% p=0.007). Seventeen of 40 (42%) neonates had electrographic seizures only: HIE (15 of 26 - 58%) versus stroke (2 of 14 -14% p=0.017). Electrographic seizures started 30 seconds or more before the onset of the clinical manifestation in 12/22 (55%) neonates with clonic seizure. The median time between electrographic onset and clinical onset in neonates with clonic seizures was 33 sec (IQR: 15-110 sec).

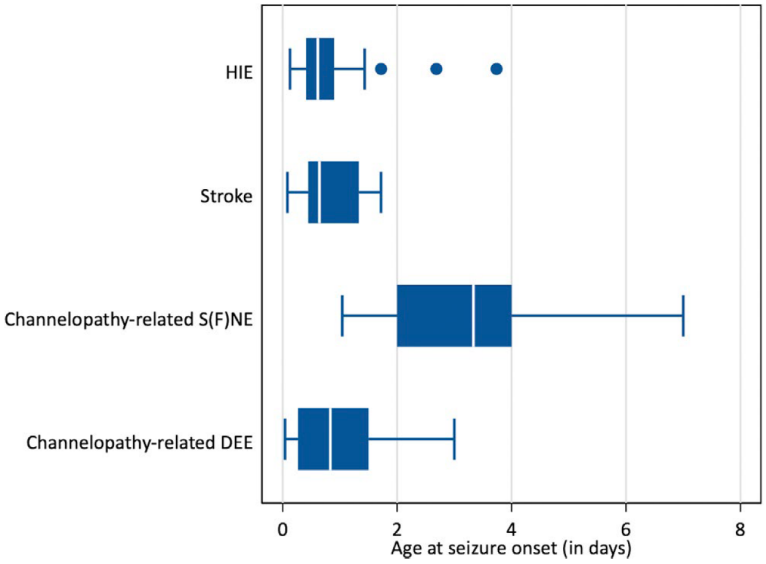
Figure 9: Most predominant seizure type based on etiology



In contrast, most infants with genetic epilepsies presented with tonic seizures (Figure 9), and all infants with tonic seizures showed clinical signs at the onset of the electrographic seizure. Eighteen of 20 (90%) neonates with genetic epilepsies had seizures characterized by an initial tonic phase with asymmetric tonic posturing. In 11 of them (61%), the tonic phase was followed by unilateral or bilateral clonic jerks representing sequential seizures. The remaining two infants with genetic epilepsies, both with *BRAT1* pathogenic variants, had myoclonic seizures. The tonic phase was followed by clonic jerks of the extremities in 11 of 18 (61%) patients with tonic seizures. The detailed clinical presentation of infants with genetic epilepsies is presented in Annex 1. All neonates with genetic epilepsies had a clinical component associated with their seizures prior to treatment, and most continued to have clinical features after treatment initiation. In Annex 2, you will find a description of the distinct genetic pathogenic variants identified in each patient. Seizure duration in neonates with acute provoked seizures tended to be longer (median: 268 seconds; IQR: 120-622s) than in neonates with genetic epilepsies (median: 66 seconds; IQR: 38-103s - $p<0.001$). Furthermore, profound and prolonged background depression following the seizure was seen in 14 of 15 (93%) infants with variants in *KCNQ2, KCNQ3,* and *SCN2A*, independently of the seizure duration and the severity of the disease. This profound attenuation was longer than the seizure itself in 13 of 14 (93%) patients (Table 7). This background depression was not seen in infants with acute provoked seizures even after prolonged seizures nor in those with *PRRT2, KCNT1,*

or *BRAT1* pathogenic variants. Seizure onset was before 48 hours of life in eight of 20 (40%) infants with genetic epilepsies compared to 38 of 40 (95%) of infants with acute provoked seizures ($p<0.001$). Only two neonates with acute provoked seizures had a seizure onset after the first 48 hours of life. One participant had a severely and diffusely depressed EEG background with seizure onset after rewarming, while the other participant had a single isolated 3-minute clonic seizure at 64 hours of life during therapeutic hypothermia, which went untreated. The patient was monitored for an additional 48 hours, and seizures did not recur. Among neonates with genetic epilepsies, 18 of 20 (90%) had a confirmed channelopathy. Eight out of 18 (44%) had a seizure onset in the first 36 hours of life, and among them, only one reached age-appropriate milestones at 3 months of life. Among the remaining 10 with a seizure onset at or after 2 days of life, nine (90%) had a normal early neurodevelopment outcome at 3 months of age. The patients with *BRAT1* pathogenic variants had a seizure onset after two weeks of life. Details regarding age at seizure onset according to etiology are provided in Figure 10.

Figure 10: Age at first seizure depending on seizure etiology



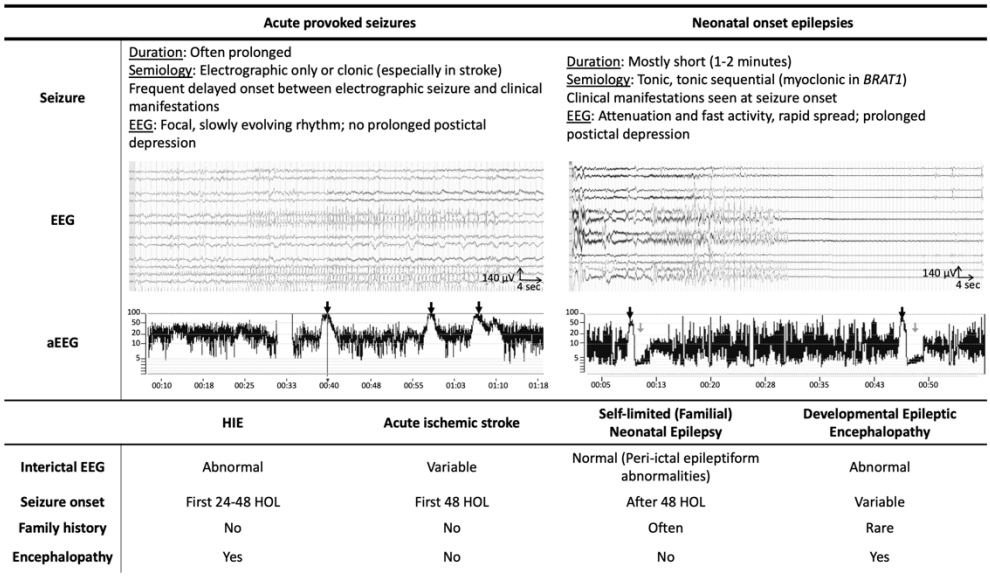
All but one neonate with genetic epilepsy and normal neurological examination at 3 months had a positive family history of neonatal seizure. All infants with acute provoked seizures and 16/20 infants with epilepsy underwent MRI within a week

from the onset of symptoms. Four infants with epilepsy and positive family history suggestive of self-limited familial neonatal epilepsy only underwent head ultrasound (Annex 1). In terms of treatment, five out of 40 (13%) neonates with acute provoked seizures either were not treated or responded to a single administration of benzodiazepines. Of the remaining 35 neonates who received ASMs, 20 (57%) responded to phenobarbital loads of 20-40 mg/kg, one (3%) responded to phenytoin/ fosphenytoin load 20 mg/kg, and one (3%) to levetiracetam 50 mg/kg as a first line ASM. Among the thirteen of 40 (33%) who required more than one ASM, all received both phenobarbital and phenytoin/ fosphenytoin, and one also received levetiracetam and a midazolam infusion. Among the neonates with genetic epilepsies, 18/20 (90%) had tonic seizures, all associated with a channelopathy. Fourteen of 18 (78%) received a phenobarbital load as the first line agent, which was ineffective in 13/14 (93%). Four of 18 (22%) received carbamazepine / oxcarbazepine initially and were seizure free. Ten of 18 (78%) received carbamazepine / oxcarbazepine after failure of one to six other ASMs, and nine of them were seizure free afterwards. All neonates with KCNQ2/3-related epilepsies who were trialed on carbamazepine/oxcarbazepine responded with seizure freedom. Of note, 8/14 (57%) infants received carbamazepine based on the electroclinical presentation, before the results of genetic testing were available. The four patients who were not trialed on carbamazepine/oxcarbazepine required multiple ASMs to achieve seizure freedom.

3. DISCUSSION

This observational double-cohort study across two centers aims to provide epileptologists, neurologists, and neonatologists with an accurate and practical approach to seizures in neonates and to allow the rapid recognition of neonates with genetic epilepsies from the larger group of neonates with acute provoked seizures (Figure 11).

Figure 11: Summary of the clinical and EEG features in APS and neonatal-onset epilepsies.



	HIE	Acute ischemic stroke	Self-limited (Familial) Neonatal Epilepsy	Developmental Epileptic Encephalopathy
Interictal EEG	Abnormal	Variable	Normal (Peri-ictal epileptiform abnormalities)	Abnormal
Seizure onset	First 24-48 HOL	First 48 HOL	After 48 HOL	Variable
Family history	No	No	Often	Rare
Encephalopathy	Yes	No	No	Yes

Epilepsy is complex, with multiple potential clinical manifestations, etiologies, treatment, and outcomes. In the last few years, there has been significant progress in the understanding of seizures and epilepsy in neonates, which is reflected in evolving terminology [49].

Accurate interpretation of seizure semiology in adults and children guides the workup and allows for tailored treatment. Many childhood epilepsy syndromes have highly characteristic semiological features, that epileptologists and neurologists have learned to recognize. We believe that the same approach is urgently needed for neonates. Our study shows that the seizure semiology *at onset* significantly correlates with etiology. Concordant with the recent study by Santarone *et al*. [243], we showed that focal clonic seizures are strongly suggestive of acute provoked seizures, in particular stroke. Also consistent with previous reports [4], we found a high rate of electrographic only seizures in critically ill neonates with acute provoked seizures. Indeed, nearly half of neonates with acute provoked seizures never had a clinical correlate to their seizure. This finding highlights the need for continuous vEEG monitoring in high risks neonates.

In this cohort, all neonates with genetic epilepsies had seizures with clinical correlates prior to ASMs, and more than two-third retained a clinical correlate throughout their course. For some of them, the clinical manifestations were still present after repeated loads of phenobarbital.

As suggested by a recent systematic review [237], we found that most neonates with genetic epilepsies present with tonic seizures. An exception was noted in neonates with *BRAT1*-related encephalopathy whose seizures are myoclonic [244,245]. Importantly, neonates with inborn errors of metabolism also tend to present with myoclonic seizures. Recognizing early these conditions is essential due to implications for specific therapies [232].

Minimizing delays in seizure control may contribute to better developmental outcomes [21]. In the past decade, the implementation of continuous vEEG monitoring in the neonatal ICU has helped to reduce the gap between seizure onset and treatment administration, especially for those neonates with HIE whose seizures are mainly electrographic only [246]. Nevertheless, the correct diagnosis and appropriate treatment for neonates with genetic epilepsies remain challenging. Historically, “neonatal seizures” were considered a single entity. Neonates still often receive a load of phenobarbital as first-line anti-seizure treatment by protocol regardless of seizure type and etiology, even though concerted efforts are trying to distinguish acute provoked seizures from genetic epilepsies [228]. Consistent with recent studies [247,9,183], we have shown that phenobarbital is generally ineffective in neonatal genetic epilepsies, as it resulted in persistence or early recurrence of seizures in eight of nine infants. The most recent International League Against Epilepsy (ILAE) position papers for classification of epilepsies [49,53] incorporate etiology along each stage of the classification. However, when it comes to neonates, we are a step behind, as the critical and clinically essential distinction between acute provoked seizures and seizures as a manifestation of genetic epilepsies is rarely done in the neonatal ICU [228]. Early recognition of infants with genetic epilepsies is essential, as some may obtain rapid seizure control with sodium-channel blockers, including carbamazepine, which may limit the impact of seizures on the developing brain [232]. While no ASM is approved for use in neonates, phenobarbital is widely used as a first-line treatment choice. Randomized controlled trials to study the efficacy of ASM in neonates have been very limited [232], and not stratified to evaluate efficacy in neonatal-onset genetic epilepsies, reflecting a gap in knowledge. In our cohort, all but one neonate with tonic seizures who were trialed off-label on carbamazepine or oxcarbazepine as first-, second-, or third-line therapy (n=13) responded with seizure freedom within hours. Our findings highlight the importance of correct interpretation of seizure semiology in neonates and the use of appropriate descriptive terminology. This emerging approach requires the elimination of the old monolithic notion of “neonatal seizures” as a single entity, and acknowledge that seizures in the nursery may be acute or the manifestation of neonatal genetic epilepsies[49]. Those two conditions are different, and for each there are different etiologies, including rare or ultra-rare disorders.

Although vEEG monitoring is the gold standard for diagnosing and classifying seizures in neonates, it may not be available, especially in the context of limited resources. Tonic seizures are easy to recognize at the bedside, as they are stereotyped with asymmetric tonic posturing and often prolonged enough to induce apnea and cyanosis. When witnessed or reviewed on video by experienced personnel, they may suggest a channelopathy, guide investigations, and justify a trial with oral carbamazepine [228]. The amplitude-integrated EEG (aEEG) pattern of KCNQ2-related seizures is also pathognomonic [248], reflecting quite nicely the pattern seen on the full EEG, and its observation may improve the recognition of these, not so rare, forms of genetic epilepsies.

For diagnosis with severely limited lifespan, such as BRAT1-encephalopathy, recognizing the clinical phenotype including erratic myoclonic seizures may help refocus the care, diminish the infant's suffering and support familial bereavement, rather than pursuing futile, often painful and ineffective efforts for extension of life [249]. *BRAT1* is not included in most targeted epilepsy panels, and identification of pathogenic variants in this gene requires whole exome sequencing as the associated lethal condition, an autosomal recessive disorder, is ultra-rare and has been only recently recognized [244]. Informing geneticists of the clinical phenotype may help to target variants in candidate genes, accelerate the diagnosis, and provide the family with essential information for reproductive counseling.

Our study shares limitations of retrospective studies, namely the potential for ascertainment bias. While having neonates monitored with rigorous guidelines allowed us to compare all cases optimally, the number of included neonates with genetic epilepsies is relatively low. Genetic epilepsies are rare, and good quality vEEG monitoring at the onset of symptoms is not always available. While we have 13 patients with *KCNQ2*-related epilepsies, we have only one or two patients with other genetic etiologies, and our cohort does not encompass all the known genetic etiologies of neonatal epilepsy. Neonatal epilepsies encompass a complex and nuanced range of disorders with an expected spectrum of seizure types, treatment responses and outcomes. Our data raise awareness on the importance of the recognition of the early clinical phenotype and are intended to motivate larger multi-center collaboration with detailed phenotyping extended to other etiologies of neonatal epilepsies, including brain malformations and epilepsies associated with inborn errors of metabolism.

4. CONCLUSION

Infants with neonatal-onset genetic epilepsies frequently remain undiagnosed until later in life, as the distinction between acute provoked seizures and epilepsy is seldom made in the neonatal ICU. We aimed to provide insights to help early discrimination of these different entities.

Neonates with channelopathies, including KCNQ2/3-, SCN2A-, and KCNT1-related epilepsies often have tonic or sequential seizures with a tonic component, a seizure type usually not observed in acute provoked seizures. Seizures associated with developmental and epileptic encephalopathies tended to arise in the first 48 hours of life at a time when acute provoked seizures are very common; later presentation and positive family history suggested a self-limited form. As neonatal seizures reach the mainstream [228], and their precise characterization is recognized[232], we should be able to raise the index of diagnostic suspicion and prioritize infants with rare conditions for targeted investigations.

Prompt recognition of tonic seizures in the setting of normal imaging, especially in neonates with a positive family history, may inform diagnostic testing and allow for a trial with carbamazepine/oxcarbazepine in the neonatal ICU, while waiting for genetic testing confirmation, avoiding ineffective and potentially harmful medications. This approach can also reduce unnecessary diagnostic procedures and their associated costs, and early enrollment in clinical research protocols to evaluate precision medicine therapies.

Finally, a definite diagnosis allows families of neonates with rare genetic epilepsies and encephalopathies to receive personalized counseling and find support from others with the same conditions, even when separated by great distances.

PART C: ULTRA-RARE GENETIC EPILEPSIES: *BRAT*1-ASSOCIATED EPILEPTIC ENCEPHALOPATHY

As partially discussed earlier, *BRAT1* pathogenic variants have been recently described to cause a spectrum of neurodegenerative conditions ranging from lethal neonatal rigidity and multifocal seizure to a less severe late-onset presentation associated with severe developmental delay, ataxia/apraxia, and cerebellar malformation [213,217,250].

On the severe part of the spectrum, neonatal cases have been associated with severe hypertonia, encephalopathy, myoclonic jerks, and plateauing head circumference leading to microcephaly. In this severe early onset part of the spectrum, all were noted to have profound neurodevelopmental delay requiring tube feeding and progressive apnea requiring either intubation or tracheostomy [212,217,218,251]. Some patients also had dysmorphic features (large front, hyperthelorism, microcephaly, long smooth filtrum) [217,252]. Their brain MRI was either normal or showed signs of delayed myelination, global or cerebellar atrophy. Infants invariably progressed to bouts of apnea and bradycardia leading to cardiac arrest and death.

The pathophysiologic mechanism of *BRAT1*-related disease remains unknown. *BRAT1* gene (Breast Cancer 1-associated ataxia telangiectasia mutated activation-1 protein) encodes a protein that interacts with the tumor suppressor BRCA1 and binds to ATM1 [253]. This phenomenon leads to DNA damage repair. In pathology studies both in human and animal models, the loss of functional BRAT1 resulted in increased apoptosis, progressive atrophy, and neuronal cell loss [220,254].

Early diagnosis of *BRAT1*-related disease is essential to avoid unnecessary diagnostic testing and allows for timely family-centered management approaches. Here, we report on four unrelated neonates with pathogenic variants in *BRAT1.* These cases bring detailed information about the very early presentation of *BRAT1* related disease and describe a common reason for delayed diagnosis of this severe disease, leading to unnecessary clinical testing and treatment.

1. CASE SERIES

This is a case series of neonates with pathogenic variants in BRAT1. Cases were identified and collected by clinicians and reviewed for the purpose of the study. Informed consent for chart review and publication of individual patient data was obtained from each family.

We identified four neonates with *BRAT1* pathogenic variants on genetic testing. The early clinical presentation was extracted at each study site. Two neonatal neurophysiologists reviewed the EEG from all cases. A pediatric neuro-radiologist reviewed all MRIs. For one case, an autopsy was performed and read by a pediatric anatomo-pathologist.
Three cases were diagnosed by whole exome sequencing on the individual and their parents (Exome trio). One case was identified on whole exome sequencing without parental sample. Exomes were performed as part of the clinical care, and pathogenicity was determined by the genetic laboratory. The genetic variants and interpretation of the finding are provided for each patient in Table 8.

Table 8: Genetic variants observed in the cohort

	Zygosity	Variant description	Type	Origin	Clinical reports (ClinVar, OMIM)	Variant classification
1	Compound heterozygous	c.1203_1204delTG c.431-10_431-7delCCCTinsTGGGTAGGG	Deletion nonsense Deletion	Mother Father	Reported rs773772842 Novel	Pathogenic variant Likely pathogenic variant
2	Compound heterozygous	c.638dup c.2005C>T	Duplication frameshift Missense	Mother Father	Reported rs730880324 Reported rs886039312	Pathogenic variant Likely pathogenic variant
3	Homozygous	c1313_1314del	Deletion	Untested	Novel	Pathogenic variant
4	Homozygous	c.2199C>A	Nonsense	Untested	Novel	Pathogenic variant

All patients were admitted at birth or shortly thereafter to the intensive care nursery for respiratory distress, abnormal movement or severe hypertonia. They all had an initial EEG, or aEEG, with a normal background. Individual clinical presentation is described in Table 9.

Figure 12 illustrates the severe hypertonia with extended feet leveling off the bed, flexed elbows, and clenched fists. To date we only have a phenotypic description in one patient who had hypertelorism, posteriorly rotated ears, microretrognatia, and short and narrow philtrum. Further analyses are needed to assess if all patients with *BRAT1* mutations have a distinctive phenotype.

Figure 12: Severe hypertonia

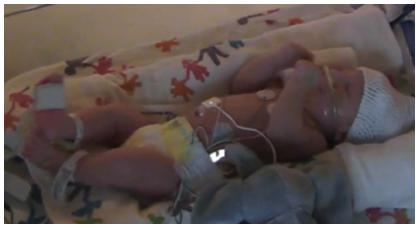


Table 9: Clinical characteristics and timeline of the 4 reported cases.

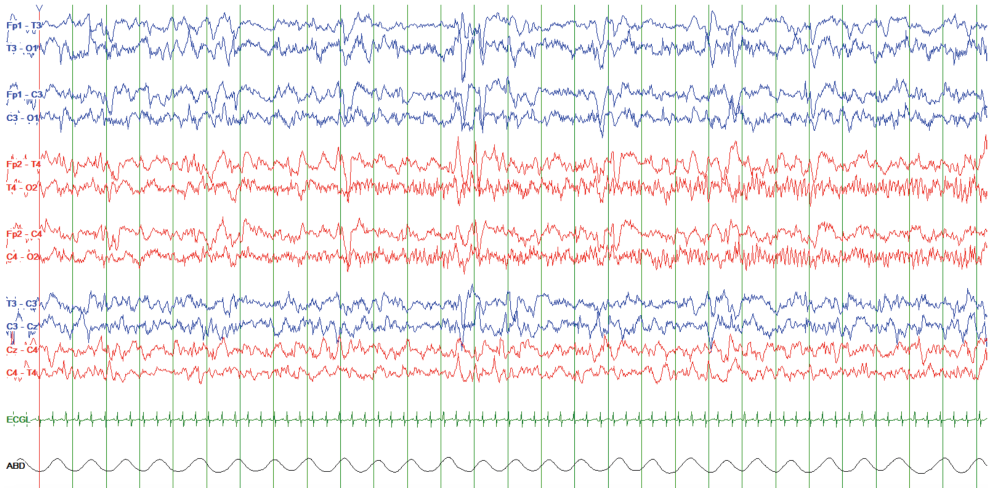
	Case 1	Case 2	Case 3	Case 4
Gestational age **HC** **Sex**	40w1d 32 Female	36w6d 32 Male	40w1d 33 Male	38w 33.5 Male
Ethnicity	European- Native American - Mexican	European	Mexican	European
Family history	None	None	None	None
Clinical finding at birth	Respiratory distress, dysmorphic features, hypertonia, hyperreflexia, sub-continuous non-epileptic myoclonic jerks.	Diffuse hypertonia and erratic sub-continuous myoclonic jerks	Hypertonia, numerous myoclonic jerks, apnea	Hypertonia, poor sucking and extreme rigidity. Multiple myoclonic jerks worsened by stimulation
EEG/aEEG recording evolution	Normal aEEG on DOL1; Numerous multifocal seizures and rhythmic paroxysmal fast activity with posterior predominance on a continuous background on DOL20	Normal background and no seizure. Seizure onset DOL15 (multifocal seizures on normal background)	EEG DOL9-12: temporal sharps, continuous background, no seizure EEG DOL30: single 10 sec seizure	EEG: slow organized background (dysmature) From 3DOL: Multifocal myoclonic seizures
Seizure semiology	Clusters of myoclonic seizures (shoulder shrugging, facial pulling and eye closure), ictal apnea or no clinical correlate.	Myoclonic seizures and subclinical seizures	Myoclonic jerks	Myoclonic jerks
AED, efficacy and tolerance	Seizure refractory to phenobarbital, levetriacetam, topiramate, and ketogenic diet	Responded initially to levetiracetam then refractory to ASM	Clonazepam, phenobarbital and levetiracetam became refractory at 2.5MOL	Responded to phenobarbital initially
Imaging	MRI: Microcephaly and poor cerebral development without any focal or specific findings	Normal except microcephaly	MRI: Normal	MRI: Normal except microcephaly
Evolution	Profound encephalopathy, no eye contact, and no feeding ability. Nl Liver enzymes at 1.5MOL.	Profound encephalopathy, acquired microcephaly	Profound encephalopathy, no eye contact, and no feeding ability.	Acquired microcephaly. Progressive apnea and bradycardia
Death	2,5 MOL (apnea and bradycardia)	2 MOL (Apnea and bradycardia) Normal liver enzymes	3 MOL (apnea and bradycardia) NB: No labs in UCSF	2y6mo

HC: Head circumference; ASM: Anti-seizure medication; DOL: Day of life ; MOL: Months of life

Three out of four (75%) were diagnosed by the referring team as having hyperekplexia, due to hypertonia and exaggeration of myoclonic jerk in response to touch or auditory stimulation. All were trialed on clonazepam which did not result in significant improvement. Two of them initially had a hyperekplexia panel

sent, delaying the final diagnosis. By one month of life, the non-epileptic myoclonic jerks were very frequent in all patients. They all had multifocal seizures despite the persistence of a normal background, as illustrated in Figure 13.

Figure 13: Right occipital seizure arising on a normal background.



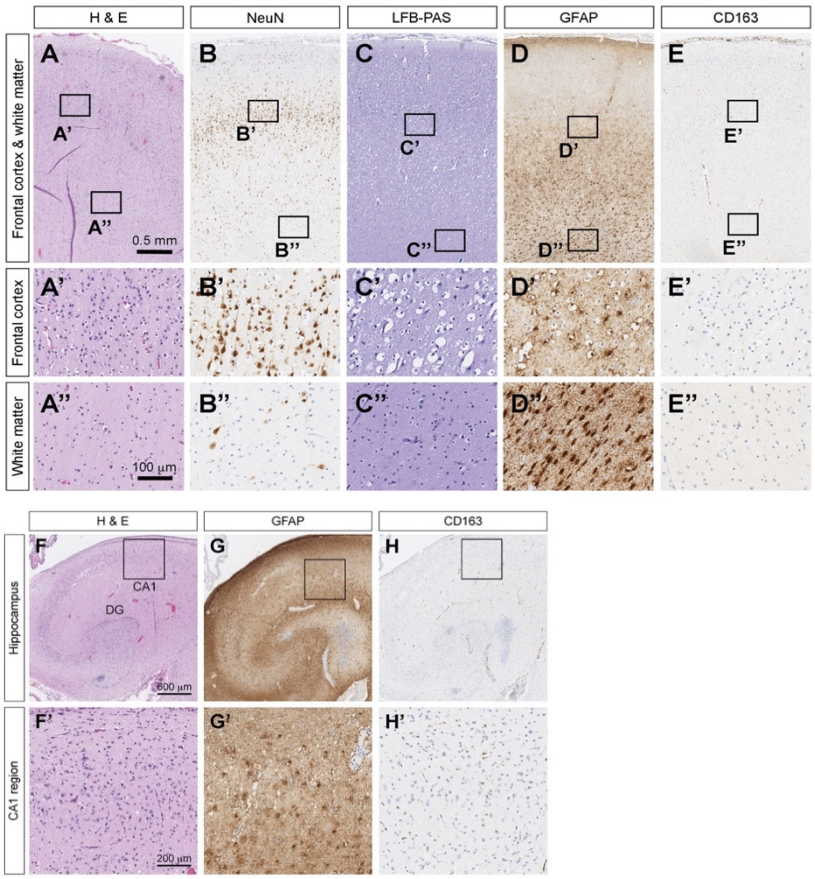
EEG subsequently worsened with the emergence of multifocal spikes or burst suppression and multifocal seizures refractory to multiple anti-seizure medications (ASM). Seizures were either myoclonic, clonic, or ictal apneas. However, most apneic episodes had no EEG correlate. One infant (case 2) was treated with valproic acid with temporary improvement in seizure burden and good hepatic tolerance.

All infants had arrested head growth and virtual absence of neurological development. They all required enteral feeding. They died between 1 and 3.5 months of life, except one patient who died at 2.5 years old, following redirection of care, in the setting of progressive refractory apnea and bradycardia.

One infant (Case 1) underwent an autopsy. The brain findings were significant for microcephaly (2/3 of expected volume) with a normally gyrated brain. Early closure of the anterior fontanelle and posterior slanting of the forehead were identified. Additionally, microscopic analysis of the brain showed severe astrogliosis and a marked loss of neurons throughout the brain with delay in myelination and loss of myelinated axons (Figure 14).

A related abnormality was neurogenic changes in the skeletal muscle of the thigh. No mitochondrial dysfunction was observed as evidenced by normal NADH, SDH, and combined COX/SDH staining.

FIGURE 14: Severe loss of neurons and astrogliosis



(A) Hematoxylin-and-eosin (H & E) sections show indistinct grey-white junction with reduced neuronal density in the frontal cortex (A'). The subcortical white matter shows a modest increase in astrocytes and decrease in oligodendroglia (A'').
(B) Immunohistochemical stain for neuronal marker NeuN confirms a significant loss of neurons most severely affects the deeper layers of the frontal cortex (layers 5 and 6)(B'). Scattered NeuN-positive neurons are present in the white matter near the grey-white junction (B'').
(C) LFB-PAS stain shows marked reduction in myelination in the white matter (C''). In addition, this stain also highlights many cortical neurons with prominent vacuoles (C').
(D) Immunohistochemical stain for GFAP shows severe astrogliopathy with profound and diffuse astrogliosis throughout the subcritical white matter. The astrogliosis appeared to be extending into the deeper layers (layers 5 and 6) of the cerebral cortex but sparing the more superficial layers (layers 1-3)(D'). The morphological features of the astrocytes were characterized by abundant cytoplasm and prominent astrocytic processes (D'').
(E) Immunohistochemical stain for CD163 shows a distinct lack of microglia in frontal cortex (E') and white matter (E''). (F) Hematoxylin-and-eosin sections show reduced neuronal density in the CA1 region of the hippocampus (F'). (G) Immunohistochemical stain for GFAP shows severe astrogliosis in the CA1 region of the hippocampus (G'). (H) Immunohistochemical stain for CD163 shows a very modest increase in microglia in the CA1 region of hippocampus (H').

2. DISCUSSION

Lethal Neonatal Rigidity and Multifocal Seizure Syndrome (OMIM# 614498) has been described in association with *BRAT1* pathogenic variants for the first time by Puffenberger *et al.*, in 2012 [255]. Since then, a few case reports have been published, and *BRAT1* mutations have been associated with a spectrum of diseases ranging from the severe neonatal form with early lethality to a less severe late-onset presentation associated with severe developmental delay, ataxia/apraxia, and cerebellar malformation [213,217,250]. In this case series, we describe four neonates with a severe early presentation, 3 of whom were referred to a tertiary center with a diagnosis of suspected hyperekplexia, delaying accurate diagnosis. Hyperekplexia shares some common features with neonatal *BRAT1*-related disease: a normal EEG at onset, associated with hyperreflexia, and exaggerated myoclonic jerks during stimulation.

However, in hyperekplexia, in contrast to *BRAT1,* neonates do not have encephalopathy and improve gradually. Developmental outcome of children with hyperekplexia is generally good, and treatment with clonazepam decreases the severity of the jerks. Other genetic mutations can be responsible for hyperekplexia-like disease: *KCNQ2* gain of function mutation [177], ponto-cerebellar hypoplasia type 4 (due to *TSEN54* pathogenic variants), and EIEE8 (Hyperekplexia and epilepsy due to *ARGEF9* pathogenic variants). Their differential diagnosis is described in Table 10.

Table 10: Conditions mimicking *BRAT1* related disease

Condition	Gene(s)	Clinical presentation
Hyperekplexia (AR/AD)	GLRA1 GLRB SLC6A5	Hypertonia and exaggerated startle in neonates associated with frequent myoclonic jerks. Respond well to Clonazepam. Improve by the end of 1st year. Sometimes mild motor delay. Normal cognitive development, no microcephaly.
Pontocerebellar hypoplasia type 4 (AR)	TSEN54	Severe neonatal encephalopathy, microcephaly, myoclonus, and muscular hypertonia. Seizure in neonatal period have been described.
Hyperekplexia and epilepsy (XLR)	ARHGEF9	Cyanosis and hypertonia at birth then tonic seizures provoked by tactile stimulation/emotion. Psychomotor arrest followed by regression and early death during childhood.
***KCNQ2* gain of function (AD)**	KCNQ2	Profound neonatal encephalopathy, nonepileptic myoclonus and severe neurodevelopmental delay, leading to early death.

Early accurate diagnosis is of critical importance as it allows informed management decisions. For example, the burden of complex surgeries (G-Tube, tracheostomy), repeated EEG, and MRI can be avoided. It also allows for genetic counseling of the infant's parents.

More recently, infants with *BRAT1* pathogenic variants have been described with epilepsy of infancy with migrating focal seizures (EIMFS) [245,256]. This rare, devastating developmental and epileptic encephalopathy is characterized by seizure migration between cerebral hemispheres. While presentation in our cases shared many features in common with EIMFS, neonates with *BRAT1* pathogenic variants had multifocal rather than truly migrating seizures.

To our knowledge, this is the first time a continuous normal voltage EEG background has been described in neonates with the most severe form of *BRAT1*-related encephalopathy. In previous case reports, neonates who presented with a severe form of the disease all presented with an abnormal EEG background: burst suppression pattern [212,213] or background slowing and multifocal spikes, spike waves, or sharp waves [214,215,255]. In one previously described case, evolution to a background with multifocal sharp waves and a focal electrical status (epilepsia partialis continua) was reported. In contrast, the early EEG recording from two of the neonates reported here showed a continuous EEG or aEEG background initially. The patients had numerous myoclonic jerks without EEG correlates in the first few days of life. By the third week of life, the EEG evolved into multifocal seizures in one case and multifocal sharp waves in the other.

The term epilepsia partialis continua is used to describe a variant of simple focal motor status epilepticus in which frequent repetitive muscle jerks, usually arrhythmic, continue over prolonged periods of time [257]. Both myoclonic jerks and epilepsia partialis continua can orient toward a diagnosis of mitochondriopathy. This could be consistent with mitochondrial dysfunction observed in some *BRAT1* deficient cells [216,258]. Hence, the clinical phenotype of these neonates shares lots of common features with mitochondrial disorders. However, hypertonia rather than the profound classical hypotonia associated with mitochondrial diseases and normal lactate/pyruvate levels were seen in all our patients. Furthermore, in our autopsy analysis, in contrast to one of the patients reported by Van de Pol *et al*., no cytochrome C oxidase deficiency was observed on muscle biopsy, and the skeletal muscle myofibers looked normal. This has clinical importance as valproic acid should be avoided in patients with mitochondrial dysfunction. In our series, one patient was treated with valproic acid with good tolerance (normal liver enzyme) and a significant reduction in the number of myoclonic jerks suggesting that mitochondrial function might not be affected in all individuals with *BRAT1*-related disease.

In our first case, the autopsy finding confirmed the microcephaly with normal gyration, implicating that brain growth rather than brain structure is significantly affected by *BRAT1* mutations. We hypothesize that the severe and disproportionate astroglial pathology cannot be entirely explained by hypoxic-ischemic encephalopathy alone but rather that the *BRAT1* mutation by itself could directly contribute to the diffuse and profound astrogliosis due to defects in cell proliferation and cell gate in the glial lineages. Further supporting this hypothesis is the distinct relative lack of microglia (normal number of macrophages). In our patient, in addition to the diffuse lack of neurons, there was a lack of axons suggesting inadequate interneuron communication. This lack of axons induced neurogenic changes on the muscle biopsy.

In conclusion, *BRAT1*-related disorders can present in neonates with a phenotype easily misdiagnosed as hyperekplexia. WES or panel including *BRAT1* gene should be performed as a priority in neonates with hypertonia, frequent spontaneous and provoked myoclonic jerks, and encephalopathy. Mitochondrial function can be normal, and valproate might be temporarily safe and effective to decrease seizure burden and improve comfort. The early normal EEG and head circumference allow for the hope that very early targeted treatment of these infants could prevent the current dreadful evolution.

CONCLUSIONS AND MOVING FORWARD

The neonatal period is the period with the highest risk of seizure in the entire life [1]. While acute provoked seizures are common, infants with neonatal-onset genetic epilepsies frequently remain undiagnosed until later in life. The distinction between acute provoked seizures and epilepsy is seldom made in the neonatal ICU. In this work, we showed how a collaborative approach, such as the one proposed in an increasing number of centers implementing a Neurologic Neonatal Intensive Care Unit [13–15], can move the field of seizures in neonates forward. The increased availability of continuous video EEG monitoring and enhanced training of the bedside team will allow for the accurate diagnosis of seizures. Specific clinical patterns can be identified that should lead to prompt targeted diagnostic testing and treatment. For example, neonates with tonic seizures should be tested for genetic diseases, and some neonates with a typical history and presentation for benign familial epilepsies could even be treated with targeted therapies (i.e. carbamazepine) even before the results of the genetic testing are available. Similarly, neonates with refractory seizures should be trialed on Pyridoxine and/or pyridoxal-5-phosphate as pyridoxine-dependent epilepsy is a treatable disease that will not respond to classical ASM. For neonates born in centers without a neuro-intensive care nursery, the focus should be on recognizing at-risk neonates and early transfer to centers of references where they can benefit from the experience of a team of providers [7], as promoted by European reference networks [259]. These centers may offer their expertise via telehealth as a first-line approach for neonates born in remote hospitals [19,259] [17,261].

As seizures in neonates become increasingly recognized as a symptom with various etiologies, we should be able to identify early neonates with suspicious phenotypes and prioritize them for targeted investigations. However, an accurate phenotypic description of the seizure and the associated signs and symptoms is often lacking from large datasets based on Electronic Medical records. These infants' phenotypes should be described accurately and in a standardized way using human phenotype ontology vocabulary [260,261] to facilitate the comparison of cases across centers and enable the analysis of large-scale data collected during routine clinical and EEG encounters. Indeed, given the rarity of each neonatal-onset epilepsy, collaboration across borders and oceans will be crucial to moving the field forward. The growth of the European reference Networks [262] and the creation of European consortiums, such as the "Solve-RD - solving the unsolved rare diseases," are promising for the future of the field. Solve-RD is a research project funded by the European Commission aiming to diagnose many rare diseases, for which a molecular cause is not known yet. This

program will use sophisticated combined omics approaches and implement a "genetic knowledge web" based on shared knowledge about genes, genomic variants, and phenotypes [261,263,264]. Accurate phenotypic data will be pooled together in the large dataset to improve our understanding and management of patients with rare diseases.

This approach will ultimately shorten the diagnosis odyssey, reduce unnecessary diagnostic procedures, and enable early enrollment in clinical research protocols to evaluate precision medicine therapies. A rapid definite diagnosis will also allow families of neonates with rare genetic epilepsies and encephalopathies to receive personalized counseling and find support from others with the same conditions, even when separated by great distances.

ANNEXES

ANNEX 1: CLINICAL PRESENTATION, MANAGEMENT AND OUTCOME OF THE 20 NEONATES WITH GENETIC EPILEPSIES

Case	Sex	Gene	Family history *	EEG background	Age at seizure onset	Seizure semiology	Seizure duration at onset and duration of background depression	Seizure frequency*	Treatment	Imaging	Seizure at 3 months	Neuro exam at 3 mo
1	F	KCNQ2	No	Lack of organization and physiologic features with almost-continuous multifocal epileptiform abnormalities intermixed with random asynchronous attenuations	72 HOL	Asymmetric tonic posturing, sometimes with paroxysmal bradycardia and desaturations. Sometimes can have bilateral tonic posturing of upper and lower extremities	30 sec followed by 90 sec depression	35/h	PB, LEV, TPM, VGB, CLB, CZP, KD, B6, PLP, B6, CBZ 20mg/kg/d (effective)	MRI mild hypomyelinization at 20 DOL and thin corpus calosum	Seizure free on CBZ	No eye tracking, axial hypotonia and opistotonos.
2	M	KCNQ2	No	Multifocal epileptiform abnormal activity with random attenuation	36 HOL	Asymmetric tonic posturing sometimes followed by unilateral or bilateral clonic jerks of upper and or lower extremities	50 sec followed by 60 sec depression	1/h	PB, LEV, B6, CBZ 20mg/kg/d (effective)	MRI Mild thinning of the corpus callosum	Seizure free on CBZ	Eye tracking but axial hypotonia and irritability
3	F	KCNQ2	No	Multifocal spikes and random attenuation	28 HOL	Ictal cry followed by asymmetric tonic posturing sometimes followed by clonic jerks.	55 sec followed by 90 sec depression	1/h	PB, CBZ 15mg/kg/d (effective)	MRI Normal	Seizure free on CBZ	No eye tracking, axial hypotonia
4	F	KCNQ2	Yes	Occasional central interictal spikes and otherwise normal background	25 HOL	Asymmetric tonic posturing followed by clonic jerks	100 sec followed by 120 sec depression	3/d	PB, LEV, CBZ 10mg/kg/d (effective)	MRI Normal	Seizure free on CBZ	Normal
5	M	KCNQ2	Yes	Normal background	80 HOL	Asymmetric tonic posturing followed by clonic jerks	35 sec followed by 190 sec depression	3/d	CBZ 10mg/kg/d (effective)	HUS Normal	Seizure free on CBZ	Normal
6	F	KCNQ2	Yes	L>R centrotemporal sharp waves and otherwise normal background	77 HOL	Asymmetric tonic posturing followed by clonic jerks	65 sec followed by 100 sec depression	2/d	CBZ 10mg/kg/d (effective)	HUS Normal	Seizure free on CBZ	Normal
7	M	KCNQ2	No	Multifocal epileptiform abnormalities with random attuation, alternating with periods of asynchronous and asymmetric burst-suppression	10 HOL	Asymmetric tonic posturing with desaturation and tachycardia	22 sec followed by 180 sec depression	4/h	PB, LEV, CBZ 20 mg/kg/d (effective)	MRI Normal	Seizure free on CBZ	Poor eye tracking, axial hypotonia
8	F	KCNQ2	No	Multifocal epileptiform abnormalities with random attenuation	12 HOL	Asymmetric tonic posturing with desaturation	25 sec followed by 50 sec depression	1/h	PB, LEV, LTG, VPA, KD, OXC 35mg/kg/d	MRI Normal	Seizures free on OXC	Poor eye tracking, axial hypotonia

Case	Sex	Gene	Family history*	EEG background	Age at seizure onset	Seizure semiology	Seizure duration at onset and duration of background depression	Seizure frequency*	Treatment	Imaging	Seizure at 3 months	Neuro exam at 3 mo
									(effective)			
9	M	KCNQ2	Yes	Normal	96 HOL	Focal tonic with apnea and desaturation	83 sec followed by 90 sec depression	3/d	PB, PHT, B6, LEV (partially effective 40 mg/kg/d)	MRI Normal	1-2/m on LEV	Normal
10	F	KCNQ2	Yes	Central spikes and sharp and otherwise normal background	80 HOL	Focal tonic with apnea and desaturation followed clonic phase	60 sec followed by 90 sec depression	3/d	CBZ 15 mg/kg/d (effective)	HUS Normal	Seizure free on CBZ	Normal
11	M	KCNQ2	No	Normal	48 HOL	Focal tonic, perioral cyanosis, desaturation 60%, followed by bilateral clonic jerks	60 sec followed by 60 sec depression	1-2/d	PB , B6, PHT 20 mg/kg/load (effective), LEV 20 mg/kg/d (effective)	MRI Normal	Seizure free on PB and LEV	Normal
12	M	KCNQ2 TSC2	No	Initially normal, then multifocal spikes with attenuation in setting of multiple anti-seizure medications	36 HOL	Focal asymmetric tonic with alternating laterality, perioral cyanosis, bilateral clonic jerk, tachycardia 220 bpm followed by marked bradycardia 60 bpm in the immediate post-ictal phase	110 sec followed by 120 sec depression	4-5/d	PB, PHT, LEV, B6, VGB, MDZ, CBZ 15 mg/kg/d (effective)	MRI Normal	Seizure free on CBZ	Mild axial hypotonia
13	F	KCNQ3	Yes	Normal background	5 DOL	Focal tonic with hyperextension limbs, eyes and head deviation, desaturation followed by bilateral lower limbs clonus	120 sec, no depression	1-2/d	PB, B6, LEV 20 mg/kg/d (partially effective), CBZ 11 mg/kg/d (effective)	MRI: focal diffusion anomalies in R BG	Seizure free on CBZ	Normal
14	F	KCNQ3	Yes	Normal background	96 HOL	Asymmetric tonic posturing followed by clonic jerks	70 sec followed by 130 sec depression	2/d	CBZ 10 mg/kg (effective)	HUS Normal	Seizure free on CBZ	Normal
15	M	KCNT1	No	Symmetric, theta-delta medium to low amplitude, lack of organization and physiological features, frequent multifocal epileptiform abnormalities intermixed with short period of random attenuation	1 HOL	Asymmetric tonic, eye and head deviation, deviation of the angle of the mouth associated with apnea and desaturation	60 sec, no depression	2/h	PB, TPM, CLN, PHT, B6, KD, LEV, pure cannabidiol (Epidiolex), potassium bromide	MRI Normal	Daily seizures on PB and potassium bromide	No eye tracking, axial hypotonia
16	M	PRRT2	Yes	Normal background	7 DOL	Asymmetric tonic posturing, at times associated with ipsilateral or contralateral eye deviation, oral automatism, and occasionally followed by hemiclonic jerking	90 sec, no depression	5/h	PB, CBZ 10mg/kg/d (effective)	MRI Normal	Seizure free on CBZ	Normal
17	F	SCN2A	No	Burst-suppression pattern or	3 HOL	Eye deviation, lip	20 sec followed by	10/h	PB, LEV, B6, TPM,	MRI Normal	Weekly	No eye tracking,

Case	Sex	Gene	Family history *	EEG background	Age at seizure onset	Seizure semiology	Seizure duration at onset and duration of background depression	Seizure frequency*	Treatment	Imaging	Seizure at 3 months	Neuro exam at 3 mo
				multifocal epileptiform abnormalities with a lack of normal named patterns in the setting of PB		smacking/chewing, followed sometimes by tonic extension of the extremities, then a brief self-resolving desaturation	30 sec depression		CLB (partially effective), CBZ 40mg/kg/d (partially effective-weekly seizures)		seizure on CBZ and CLB	axial hypotonia, intermittent posturing
18	M	SCN2A	Yes	Normal background	48 HOL	Focal with initial hypertonia followed by hypotonia and cyanosis	110 sec followed by 50 sec depression	2-3/d	PB, VPA 30 mg/kg/d and VGB 100 mg/kg/d (partially effective)	MRI Normal	Seizure free on VPA and VGB	Normal
19	M	BRAT1	No	Bilateral frontal epileptiform abnormalities and otherwise normal background	17 DOL	Multifocal myoclonic seizures at presentation, focal clonic seizures involving face and limbs with alternating side appeared at four weeks of age	20 sec	10/d	LEV, CBZ, PHT, B6, PB, P5P, MDZ, ZNS, PHT, VPA.	MRI Normal	Refractory before death	Diffuse hypertonia. No milestones reached, died at 66 DOL
20	F	BRAT1	No	Frequent runs of rhythmic sharpened delta and frequent multifocal sharp waves and otherwise normal background	23 DOL	Multifocal myoclonic seizures at presentation, multifocal clonic seizures appeared at tree weeks of age	30 sec, no depression	5/h	CZP, B6, B9, PB, LEV, CLB, FOS, P5P, TPM, MDZ	MRI microcephaly	Refractory before death at 2.5mo	Diffuse hypertonia. No milestones reached, death at 69 DOL

ANNEX 2: Details of diagnostic genetic results for neonates with genetic epilepsy

Cases	Gene	UniprotKB identifier	NCBI identifier	Coding sequence variant	Protein variant	Type	Zygocity	Origin	Clinical reports (ClinVar, OMIM)	Impact prediction (polyphen2)	Minor allele frequency (ESP)	Mode of inheritance	Variant classification	Other comments	Diagnostic test
1	KCNQ2	O43526	NM_172107.2	c.1734 G>C	p.M578I	Missense	Heterozygous	De novo	Reported rs796052655 [8]	1	0	AD	Pathogenic	SCN1A c.3405 A>G (VUS Mat inherited) TSC2 c.5095 G>A (VUS paternal Inherited)	Targeted NGS gene panel
2	KCNQ2	O43526	NM_172107.2	c.841 G>T	p.G281W	Missense	Heterozygous	De novo	Reported rs794727813 [183]	1	0	AD	Pathogenic	Compound heterozygous RTTN (c.1921A>G and c.3581A>G)	WES
3	KCNQ2	O43526	NM_172107.2	c.683 A>G	p.H228R	Missense	Heterozygous	De novo	Published [265]	1	0	AD	Likely pathogenic		Targeted NGS gene panel
4	KCNQ2	O43526	NM_172107.2	c.619 C>T	p.R207W	Missense	Heterozygous	Unknown	Published [266,267]	1	0	AD	Pathogenic		Targeted NGS gene panel
5	KCNQ2	O43526	NM_172107.2	c.807 G>A	p.W269X	Nonsense	Heterozygous	Inherited	Published [268]	N/A	0	AD	Pathogenic		Targeted NGS gene panel
6	KCNQ2	O43526	NM_172107.2	c.807 G>A	p.W269X	Nonsense	Heterozygous	Inherited	Published [268]	NA	0	AD	Pathogenic		Single gene
7	KCNQ2	O43526	NM_172107.2	c.1749 G>C	p.K583N	Missense	Heterozygous	Unknown	Novel	1	0	AD	Likely pathogenic		Targeted NGS gene panel
8	KCNQ2	O43526	NM_172107.2	c.1088 A>G	p.Y363C	Missense	Heterozygous	Unknown	Published[269]	1	0	AD	Likely pathogenic		Targeted NGS gene panel
9	KCNQ2	O43526	NM_172107.2	c.1021 C>T	p.Q341*	Nonsense	Heterozygous	Inherited	Published[270]	NA	0	AD	Pathogenic		Targeted NGS gene panel
10	KCNQ2	O43526	NM_172107.2	c.1021 C>T	p.Q341*	Nonsense	Heterozygous	Inherited	Published[271]	NA	0	AD	Pathogenic		Single gene
11	KCNQ2	O43526	NM_172107.2	c.388 G>A	p.E130K	Missense	Heterozygous	De novo	Reported RCV000203592.1	1	0	AD	Pathogenic		Targeted NGS gene panel followed by WES
12	KCNQ2	O43526	NM_172107.2	c.1741 C>T	pR581*	Nonsense	Heterozygous	De Novo	Reported RCV000678061.1	NA	0	AD	Pathogenic	TSC2 pathogenic variant mosaiscism 2.4%	Targeted NGS gene panel

Cases	Gene	UniprotKB identifier	NCBI identifier	Coding sequence variant	Protein variant	Type	Zygocity	Origin	Clinical reports (ClinVar, OMIM)	Impact prediction (polyphen2)	Minor allele frequency (ESP)	Mode of inheritance	Variant classification	Other comments	Diagnostic test
13	KCNQ3	O43525	NM_004519.3	c.1060 G>A	p.G354R	Missense	Heterozygous	Inherited	Reported rs796052680	1	0	AD	Likely pathogenic		Targeted NGS gene panel
14	KCNQ3	O43525	NM_004519.4	c.923 G>C	p.W308S	Missense	Heterozygous	Inherited	Reported rs1064794632	0.982	0	AD	Likely pathogenic		Targeted NGS gene panel
15	KCNT1	Q5JUK3	NM_020822.2	c.776 C>A	p.A259D	Missense	heterozygous	De novo	Published[272]	0.630	0	AD	Pathogenic		WES
16	PRRT2	Q7Z6L0	NM_001256442.1	c.649dupC	p.R217fs*8	Frameshift	Heterozygous	Inherited	Published [273]	NA	0	AD	Pathogenic		WES
17	SCN2A	Q99250	NM_021007.2	c.2713 A>G	p.K905E	Missense	Heterozygous	De novo	Reported rs886043250[269]	0.998	0	AD	Pathogenic		(Single gene KCNQ2/3) WES
18	SCN2A	Q99250	NM_021007.2	c.718 G>A	p.V261L	Missense	Heterozygous	Inherited	Reported rs1064795014	0.999	0	AD	Pathogenic		Targeted NGS gene panel
19	BRAT1	Q6PJG6	NM_001350626.1	c.638dup & c.2005 C>T	p.V214Gfs*189 & p.R669W	Missense	Compound heterozygous	Inherited	Novel	NA	0	AR	Pathogenic/ likely pathogenic		WES
20	BRAT1	Q6PJG6	NM_001350626.1	c.1203_1204delTG & c.431-10_431-7delCCCTinsTGGGTAGGG	p.C401X & IVS4-10_ IVS4-7delCCCTinsTGGGTAGGG	Nonsense	Compound heterozygous	Inherited	Reported rs773772842 & Novel	NA	0	AR	likely pathogenic/ likely pathogenic		WES

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