Developmental and epileptic encephalopathies: from genetic heterogeneity to phenotypic continuum

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# Table of Contents

1. **Introduction to the Concept and Pathophysiology of Developmental and Epileptic Encephalopathy (DEE)** ................................................................. 4

2. **General Concepts of Epileptogenesis and Epilepsy** ................................................................. 13

3. **Main (Known) Determinants of Normal and Abnormal Brain Development That Influence Epileptogenesis** ............................................................................................................. 17

4. **Non-Genetic Determinants of the Epileptogenic Risk** ............................................................................................... 22

5. **Genetic Determinants of DEE: Experimental Models** .................................................................................. 23

6. **Dysfunctions in Neural Cell Migration, Proliferation, and Synaptogenesis** ........................................ 25

7. **Dysfunctions in Intrinsic Excitability** ............................................................................................... 29

8. **Dysfunctions in Synaptic Transmission and Plasticity** .................................................................................. 51

9. **Dysfunctions in Neuronal Housekeeping: MTOR and Autophagy** ...................................................... 67

10. **The Search for Personalized Treatment Approaches** ................................................................................. 73

11. **Conclusions** ............................................................................................................................................. 79
ABSTRACT

Developmental and epileptic encephalopathies are a heterogeneous group of disorders characterized by early-onset, often severe epileptic seizures, EEG abnormalities, on a background of developmental impairment that tends to worsen as a consequence of epilepsy. DEEs may result from both non-genetic and genetic etiologies. Genetic DEEs have been associated with mutations in many genes involved in different functions including cell migration, proliferation, and organization, neuronal excitability, and synapse transmission and plasticity. Functional studies performed in different animal models and clinical trials on patients have contributed to elucidate pathophysiological mechanisms underlying many DEEs and explored the efficacy of different treatments. Here, we provide an extensive review of the phenotypic spectrum included in the DEEs, of the genetic determinants and pathophysiological mechanisms underlying these conditions. We also provide a brief overview of the most effective treatment now available and of the emerging therapeutic approaches.

Keywords: developmental and epileptic encephalopathies; epileptogenesis; channellopathies; synaptopathies; personalized treatment approaches.

CLINICAL HIGHLIGHTS

Epilepsy is the third leading contributor to the global burden of neurological disorders and affects 65 million people worldwide. Based on the clinical and EEG features, etiologies, and comorbidities, different categories of epilepsy types and syndromes are recognized. Among them, developmental and epileptic encephalopathies (DEEs) represent the most severe end of the spectrum. This review will help Physiological Reviews’ readership to increase their knowledge about the different types of DEEs, their etiologies and pathophysiological mechanisms, and available and emerging treatments.
I. INTRODUCTION TO THE CONCEPT AND PATHOPHYSIOLOGY OF DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY (DEE)

Epilepsy is the third leading contributor to the global burden of neurological disorders, and affects 65 million people worldwide (1). Based on the clinical and EEG features, etiologies, and comorbidities, different categories of epilepsy types and syndromes are recognized (2). Developmental encephalopathies (DEs) are a group of severe disorders with early onset of signs of developmental impairment, associated with other neurological symptoms such as autonomic dysfunction, behavioral disorders, and motor impairment (3). In DEs, developmental delay/impairment is a prominent feature, whilst the epileptic activity (seizures and EEG abnormalities) does not appear to be causally associated with developmental delay, stagnation, or regression. Epileptic encephalopathies (EE) comprise a large, heterogeneous group of severe epilepsy syndromes characterized by several seizure types, frequent epileptiform activity on EEG, and developmental delay or regression (4). In EE, no pre-existing developmental delay is observed, and the cause of delay is attributed to an interference that epilepsy has on physiological brain processes. However, when severe epilepsy has a very early onset, it is often impossible to know whether the underlying cause of the epileptic encephalopathy would not in itself have caused developmental delay, even in the absence of epilepsy. For this reason, recent definitions refer to ‘developmental and epileptic encephalopathies (DEEs)’ to designate a heterogeneous group of disorders characterized by early-onset, often severe epileptic seizures, EEG abnormalities, on a background of developmental impairment that tends to worsen as a consequence of epilepsy (2).

This generates a complex clinical picture in which both developmental abnormalities and severe epilepsy/EEG discharges contribute to the observed impairment, each to an extent that it is hard to measure. The spectrum of DEEs by age of onset is described in **TABLE 1**, adopting the latest proposal for the classification of epilepsy syndromes by the International League Against Epilepsy.
In certain DEEs, specific gene defects may create recognizable etiology-specific syndromes, whereas, in others, a variety of genetic variants may be associated with the same epilepsy syndrome. Such genetic heterogeneity is characteristic of the infantile spasms syndrome (ISS) and Lennox-Gastaut syndromes (LGS), where genetic variants affecting distinct molecular or signaling pathways may lead to similar electroclinical syndromes (8–12). Moreover, DEEs may also result from nongenetic etiologies, including structural, toxic/metabolic, infectious, or immune, which may appear either independent of or in the setting of certain genetic etiologies (TABLE 1). Despite the advances in diagnostic tools, the underlying etiology may remain unknown in a significant portion of patients with specific syndromes. For example, in ISS, a third of the patients have unknown etiology, ~24% have genetic or genetic-structural, and almost half have structural/metabolic or other acquired etiologies (13). Similar distributions of etiologies are also encountered in Lennox-Gastaut syndrome, although, as discussed above, increasing numbers of genetic variants and associations have emerged, as use of genetic tests becomes more widespread (14).

In DEEs, the co-occurrence of epilepsy and intellectual disability (ID) can involve at least two non-exclusive mechanisms. These mechanisms include uncontrolled, frequent or prolonged seizures that can interfere with brain developing programs, resulting into inadequate construction of cortical networks and poor cognitive outcomes (15), as well as genetic mutations or adverse environmental factors that can induce both seizures and cognitive impairment. For example, genetic mutations inducing specific synaptic defects might cause seizures because of aberrant connectivity, as well as intellectual disability because of altered synaptic plasticity (16). In many DEEs, epilepsy co-exists with other comorbidities, both neurological and extra-neurological. Neurological comorbidities differ in type and severity, and span from subtle learning difficulties to
psychiatric features, such as autism spectrum disorder (ASD) or depression to psychosocial concerns (2).

In spite of their apparent phenotypic continuum, DEEs include a large collection of specific neurogenetic disorders. Several studies have been performed over the last two decades to identify molecular determinants and characterize pathophysiological mechanisms underlying DEEs, contributing to a greater understanding of their neurobiological and clinical aspects.

Next generation molecular testing has boosted gene discovery for many human disorders. According to the Online Mendelian Inheritance in Man catalogue (OMIM, https://www.omim.org), 172 genes have been identified as causative for epileptic encephalopathy and, among them, 90 have been recognized to date as a cause of DEEs (TABLE 2 and SUPPLEMENTAL TABLE S1, https://figshare.com/articles/dataset/Guerrini_et_al_Supplemental_Table_S1/19666521).

However, this list may not be exhaustive, as the concept of DEEs is wide and encompasses a large number of conditions and, in up to 8% of individuals are caused by de novo copy number variants (CNVs) (17, 18).

The study of large cohorts of affected individuals with variable but related phenotypes performed using next generation sequencing (NGS) approaches including targeted gene panels, whole exome and whole genome sequencing, has now demonstrated that 30-50% of DEEs can be attributed to de novo pathogenic variants in single genes (9, 16, 19). Low-level somatic mosaicism is observed in about 10% of parents of probands with DEEs (20, 21), with important consequences for recurrence risk estimation. In addition to single de novo variants, DEEs pathogenesis has also been associated with recessive mutations in 11-38% of patients (22, 23). Additional genetic mechanisms, that are often not detectable by standard NGS methodology, might also contribute to DEE pathophysiology, including non-exonic variants, brain mosaicism in the patient, oligogenic inheritance, and epigenetic changes.
The contribution of non-exonic variants is mainly related to ‘poison’ exons that, when spliced into an RNA transcript, trigger nonsense mediated decay (NMD), a surveillance system that detects and degrades RNAs harboring premature termination codons (PTCs) (FIGURE 1). An example of how such mechanism is associated with DEEs has been described by Carvill and collaborators who sequenced 11 non-coding candidate regions of the SCN1A gene in 640 individuals with unsolved DEE, selected based on their evolutionary conservation and functional features. The authors identified five variants in intron 20 that promoted inclusion of a “poison” exon and caused a reduction of the amount of full-length SCN1A protein (24). Since transcriptome studies on purified neural progenitor cells (NPCs) have identified hundreds of differentially-spliced exons (25), reduced gene expression due to inclusion of poison exons may represent an etiological mechanism of a broad range of neurological diseases, including DEEs.

Somatic mosaicism is the result of a variant arisen at postzygotic level, which is inherited by daughter cells via mitotic division, and results in genetically distinct subsets of cells in the same individual. Based on the timing when the variant arises, it can affect one or multiple tissues. Deep sequencing studies in dysplastic brain/blood paired samples have correlated brain-confined mutations with focal malformations of cortical development (MCDs). Mutations in the mTOR pathway genes AKT1, AKT3, DEPDC5, MTOR, NPRL2, NPRL3, PIK3CA, PIK3R2, TSC1, and TSC2 can now be considered as the major known cause of focal cortical dysplasia type II (FCDII) and hemimegalencephaly (HME) (26), while somatic mutations in SLC35A2, encoding a UDP-galactose transporter, have been identified in a limited number of patients with FCD type I, mild malformations of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE), and with non-lesional focal epilepsy (NLFE) (27–31). In addition, somatic variants - either microdeletions in SCN1A or point mutations in CDKL5, PCDH19, SCN1A, and SCN2A - have been identified in 1-3.5% of patients with DEE who were mutation-negative to single gene, multigene
epilepsy panel or whole-exome sequencing (WES) performed in a clinical diagnostic setting (32, 33). Despite this evidence, the impact of somatic mutations in DEEs is likely to be underestimated, as the major challenge in detecting causative somatic mutations is to analyze the right target brain tissue which, for many DEE patients, is not easily accessible. For this reason, recent assays developed from noninvasive protocols for tumor diagnosis and progression monitoring have been attempted to identify low level mosaicism for known or recurrent mutations associated with lesional refractory epilepsy in free DNA extracted from cerebrospinal fluid biopsies (34, 35).

Oligogenic inheritance refers to the concept that, in some cases, diseases with complex phenotypes are not inherited as simple single-gene Mendelian disorders, nor they are classic complex traits, but rather fit a model in which mutations in a small number of genes may interact genetically to manifest a phenotype (36). In oligogenic conditions, one of the genes is the major disease-causing gene, while the others act as modifiers. Some DEEs can be attributed to this type of inheritance. Indeed, various studies have demonstrated that co-occurrence of two or more mutations in distinct ion-channel genes can contribute to the epileptic phenotype in both patients and animal models. (37–42). Performing targeted and whole exome sequencing in a boy who presented with profound developmental delay, failure to thrive, ataxia, hypotonia, and tonic-clonic seizures that caused his death, Hasan and collaborators identified a pathogenic variant in KCNJ10, encoding the inward rectifying K+ channel Kir4.1 and a pathogenic variant in KCNT1, encoding Na+-activated K+ channel known as Slo2.2 or SLACK. Functional studies performed in in Xenopus laevis oocytes confirmed the functional effects of the two variants. This finding revealed that, when co-occur, pathogenic variants in Kir4.1 and SLACK result in a fatal disease (40). To gain support for the hypothesis that genetic modifiers can influence clinical presentation in patients with SCN1A-derived genetic epilepsy with febrile seizures plus (GEFS+), Hawkins and collaborators (38) combined the Scn1α-R1648H allele with either Scn2αQ54, which causes spontaneous, adult-
onset partial motor seizures or $Kcnq2^{V182M/+}$, which causes increased susceptibility to induced seizure. Double heterozygous mice exhibited early-onset, generalized tonic–clonic seizures and juvenile lethality. These results demonstrate that variants in $Scn2a$ and $Kcnq2$, can dramatically worsen the phenotype of mice carrying the $Scn1a$-R1648H mutation (38).

A digenic inheritance based on somatic mutations in two mTOR pathway genes ($mTOR$ and $RPS6$) has also been demonstrated in association with hemimegalencephaly and intractable epilepsy (43).

Aberrant chromatin states leading to altered gene expression patterns (epimutations) have been detected in several conditions. Epimutations can occur secondary to a DNA mutation in a cis- or trans-acting factor (secondary epimutations), or as “true” or primary epimutations in the absence of any DNA sequence change (44). In the brain, such alterations can impair the transfer of information that bind short-lived cellular signals to the whole neuronal activity and the global gene expression (45). Emerging findings in animal models and human brain tissue have demonstrated that DEEs could be ascribed to both classes of epimutations. A classic example of DEE associated with secondary epimutations is Rett syndrome, which can be caused by both nucleotide mutations in, and duplications of $MECP2$, encoding a methylated DNA-binding protein (46).

Alterations in DNA methylation have not been explored much in epilepsy yet, but they represent a good example of primary epimutations related to DEEs. Indeed, in the brain, this epigenetic modification regulates cell fate determination and maturation, and plays a fundamental role in the induction of activity-dependent synaptic plasticity, memory, and cognition (47–49). Dynamic alterations in DNA methylation can also contribute to epileptogenesis. For example, various studies have shown that the brain-derived neurotrophic factor (BDNF), whose expression is regulated by a variety of cellular processes, including methylation of its promoter (50), results
upregulated in areas implicated in epileptogenesis (51). In addition, in hippocampal neurons, the
inhibition of DNA methyltransferases, which mediate demethylation of the BDNF promoter,
results in the suppression of neuronal excitability and network activity (52).

Various nongenetic etiologies have also been associated with certain DEEs, including hypoxic-
ischemic encephalopathy (HIE), cerebrovascular, infectious, or autoimmune disorders, tumors,
brain trauma, or metabolic disorders (5–7, 13, 53). While the molecular pathogenesis of these
etiologies is more complex and multifactorial than genetic etiologies, they offer a setting where
questions of “common pathways or mechanisms” in disease pathogenesis, network dysfunction,
and drug-refractoriness can be explored to develop therapies with broader applications. For
example, interneuronopathies, as well as mTOR dysregulation, have been implicated in both
genetic and nongenetic etiology DEEs (54, 55).

Many pathophysiological mechanisms underlie epilepsy and cognitive phenotypes in DEEs, leading
to either dysfunction of specific cortical networks or to more generalized epileptogenic changes.
These multiple and intersecting mechanisms make genotype/functional phenotype correlations
difficult. Cortical and subcortical neuronal networks may interact with each other, and in turn, can
cause widespread functional changes in otherwise normal cortex (56). The firing of excitatory
cortical neurons is finely regulated by the interplay of sodium and potassium channel activity,
which is mediated by chemical and ionic gradients across the cell membrane. If the balance
between sodium and potassium gradients is perturbed (for example due to mutations in genes
encoding Na⁺ or K⁺ ion channels or for components of the Na⁺/K⁺ pump) (TABLE 2) abnormal
depolarization arises, which in turn causes abnormal neuronal activity and cortical excitability (57).
Altered interconnections of glutamatergic neurons are another possible cause of abnormal
depolarization and mutations in various genes encoding for glutamate receptors or carriers (TABLE
2) have been associated with different DEEs (58–63). In addition to altered firing in excitatory
neurons, epileptogenesis in DEEs may be related to dysfunctions in interneuron networks. An archetype of genetic lesions associated with such mechanism is represented by *SCN1A* mutations, which are associated with Dravet syndrome and a broad category of other epilepsy phenotypes. A series of functional studies have highlighted how changes in membrane properties of one specific cellular population result in altered neuronal network dynamics and widespread cortical dysfunction, in turn leading to an epileptic phenotype. *SCN1A* mutations are mainly associated with loss of function in a subtype of voltage-gated sodium channel, the Na\(_{\text{v}}\)1.1 channel, mainly expressed in inhibitory neurons (64). This functional alteration would be expected to predispose to decreased neuronal activity. However, *in vivo*, it is actually associated with increased epileptogenicity as it results in severely impaired sodium currents and action potential (AP) firing in gamma-aminobutyric acid (GABA)ergic inhibitory neurons, without detectable effects on excitatory pyramidal neurons (64, 65). Therefore, the epileptogenic effects of *SCN1A* mutations are primarily mediated by an altered activity of inhibitory interneurons in the cortex rather than by abnormal firing of excitatory neurons (66). Recent single cell RNAseq studies performed in postmortem adult human and rodent brain tissues have confirmed that *SCN1A* (*Scn1a*) is predominantly expressed in inhibitory neurons (67). Conversely, *SCN2A/3A/8A* (*Scn2a/3a/8a*), which also encode for voltage-gated sodium channels, are preferentially expressed in excitatory neurons in multiple brain regions, suggesting that epilepsy due to mutations in these genes is mainly associated with direct alterations of the excitatory conductance (67). Expression and *in vitro* and *in vivo* electrophysiological studies are therefore crucial to establish the functional effects of channel mutations which may differ to same genes families or, as we shall see, even with same gene.

Mutations in genes encoding glutamate receptors or ion channels can also cause epileptogenic structural brain abnormalities. Using the DREADD (designer receptor exclusively activated by a
designer drug) approach, Hurni and collaborators showed that transient embryonic activation of
migrating projection neurons (PNs) induced transcriptional changes in a variety of activity-
dependent receptors including glutamatergic metabotropic, kainate, NMDA, and AMPA receptors,
that were accompanied by premature branching and persistent laminar mispositioning of
superficial layer PNs into deep cortical layers, without affecting expression of layer-specific
molecular identity markers (68). These findings support the hypothesis that increased intrinsic
activity during migration, a condition that can be caused also by mutations in DEE-causing genes,
acts as a stop signal for migrating cortical PNs (68).

Using a similar DREADD-based approach it has been demonstrated that in the developing mouse
neocortex ventricular zone progenitors become more hyperpolarized as they generate successive
subtypes of neurons (69). Experimental in vivo hyperpolarization shifted the transcriptional
programs and division modes of these progenitors to a later developmental status, with
precocious generation of intermediate progenitors and a forward shift in the laminar, molecular,
morphological, and circuit features of their neuronal progeny (69). These findings indicate that,
during development, altered bioelectrical processes can also affect non-excitable cells, including
neuronal progenitors.

Genetic background may also alter the genotype-phenotype associations. This is well known in
humans and has also been demonstrated in mouse models from different labs. A striking example
was presented by Glasscock and collaborators whereby combination of a Kcna1 knockout and a
Cacna1a missense mutation masked the absence epilepsy associated with Cacna1a and
attenuated limbic seizures and death expected from the Kcna1-null mutation (37). The C57BL/6
background also confers a more severe phenotype in Na,1.1+/- mice with targeted deletion of the
last encoding exon, than the 129/SvJ background (70). In the C57BL/6 background, Na,1.1+/- mice
manifest hyperthermia-induced and spontaneous seizures, cognitive and behavioral deficits, early
mortality. In contrast, in the 129/SvJ background have hyperthermia-induced seizures, less severe spontaneous seizures and no cognitive deficits.

II. GENERAL CONCEPTS OF EPILEPTOGENESIS AND EPILEPSY

Epileptogenesis is the chronic process by which a brain network is functionally altered toward increased seizure susceptibility, thus having an enhanced probability to generate spontaneous and recurrent seizures. For this reason, epileptogenesis has been traditionally considered in the context of a “latent period” between the causative insult and the first clinical seizure (71). This concept, however, although applicable to several acquired conditions, especially post-traumatic, post-stroke or post-infectious epilepsy, appears to be less apt to describe what happens in the context of genetically determined DEEs where the bases for epileptogenesis are most often imbricated with the altered dynamics of brain development and neural networking. Irrespective from its causes, however, epilepsy is defined by recurrent and unprovoked seizures, and can be divided into different categories, which are defined as generalized, focal (formerly called partial), and combined generalized (57, 71), according to how the epileptogenic process is distributed in the brain. These categories are defined based on the predominant types of seizures, including generalized- or focal-onset (1, 2, 72).

A seizure can be conceptualized as the result of a distortion of the normal balance between excitation (E) and inhibition (I), resulting from factors that may alter brain function at many levels, from subcellular signaling cascades to widespread neuronal circuits (1). Genetic factors (i.e., mutations in specific genes) can affect brain function at any level, from ion channels to receptor function and synaptic connectivity. Acquired cerebral insults (e.g., stroke or traumatic brain injury) are mainly associated with alterations in circuit function (1). Excessive neuronal firing alone does not necessarily cause a seizure, which also requires synchronization of a network of neurons. Glutamatergic interconnections can promote such synchronization. Application of a pro-
convulsant agent to hippocampal slices in a feline model precipitates interictal discharges whose intracellular correlate is the paroxysmal depolarizing shift (PDS). PDS is a network-driven burst associated with a sudden depolarization of the membrane potential which lasts hundreds of milliseconds and usually triggers a series of APs on its rising phase. Since cortical pyramidal cells are richly interconnected by excitatory glutamatergic synapses, it has been proposed that the mechanism underlying PDS is a “giant” excitatory postsynaptic potential (73).

Gap junctions (GJ) are another possible promoter of neuron synchronization. These specialized intercellular connections allow a ‘low-resistance’ pathway of current flow from one cell to another which, in neurons, may determine a rapid and effective synchronization. In addition, GJ located between proximal axons of principal neurons (axon-axon gap junctions) can promote epileptogenesis by providing pathways for direct spread of APs across neurons. A by-product of such spread is the ability of axonally coupled neurons to generate oscillations at very high frequencies (≥70 Hz) (74). Seizure activity, both \textit{in vivo} and \textit{in vitro}, begins with very high-frequency oscillations, suggesting that axon-axon gap junctions may play a major role in seizure initiation (74). Besides interneuronal GJ, inter-glial GJ can also be considered an important mechanism for seizure generation (75). Studying mice with coupling-deficient astrocytes, obtained by crossing conditional connexin-43 deficient mice (connexin-43\(^{\text{Cx43fl/fl:HGFAP-Cre}}\)) with connexin-30\(^{-/-}\) mice, Wallraff and collaborators showed how gap junctions in the impaired astrocytes accelerated potassium clearance, limited potassium accumulation during synchronized neuronal firing, and reduced the threshold for the generation of epileptiform events (76). Other studies, conducted in a similar \textit{in vivo} model, demonstrated that impaired glucose metabolism through astroglial networks can contribute to epileptiform activity (77).

A third mechanism of enhanced synchronization is based on impaired inhibition. Individual GABAergic interneurons can effectively phase spontaneous firing in hippocampal pyramidal cells
due to the interaction between GABA_A receptor-mediated hyperpolarizing synaptic events and intrinsic oscillatory mechanisms in the pyramidal cells. Since interneurons make numerous connections to pyramidal cells in local areas, a single discharging interneuron can synchronously hyperpolarize a large number of pyramidal cells. Once GABAergic inhibition ceases, there is activation of voltage-dependent currents in pyramidal cells, resulting in a synchronous depolarization of a number of cells which might be high enough to trigger seizures (78).

Certain signature seizures and EEG patterns in DEEs also highlight the importance of the corticothalamic network (SWD/atypical absences, generalized seizures) or brainstem structures (tonic seizures, spasms) in the generation or development of generalized seizure activity.

The thalamus serves as a gate in trafficking sensory information to and from the cerebral cortex, and its activity is controlled by the basal ganglia (79). The sensory relay thalamic neurons (TC) form reciprocal glutamatergic connections with the cortex, but also project to the GABAergic neurons of the nucleus thalami reticularis (nRT). The nRT neurons receive excitatory input from both the cortex and TC neurons and send inhibitory projections to TC neurons (FIGURE 2A). Additional inhibitory input to the TC relay neurons is also stemming from the local GABAergic interneurons (FIGURE 2A). The SWDs are generated after a burst of excitatory postsynaptic potentials (EPSPs) from the TC neurons excite the nRT interneurons which in turn send bursts of inhibitory input to the TC neurons causing pronounced inhibitory postsynaptic potentials (GABA_A and GABA_B IPSPs, FIGURE 2A) leading to activation of hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN), and low threshold calcium channels (T) which cause a calcium spike that triggers a burst of APs. The re-excitation of TC neurons gives rise to a new cycle by eventually engaging also cortical neurons. The typical SWD in humans is ~3 Hz, whereas in models of absence and in many control rodents, typical SWD are 7-8 Hz (79).
Atypical absence seizures (AAS) are slower (<2.5 Hz in humans, usually 3-6 Hz in rodents), may have atypical morphology and may not necessarily impair awareness. AAS utilize also the corticothalamic network, although there may be more inputs from the limbic structures which result in augmented cortical excitation. Certain forms of atypical SWD may also be generated from isolated cortex or thalamus (80, 81). The utilization of existing circuitry and networks for the generation of these seizures allows for transitional states, during which SWD may arise from physiological rhythms, such as sleep spindles, when cortical neurons become hyperresponsive to the thalamocortical excitatory input (82).

While our knowledge about the functional bases of generalized epileptogenesis and seizures are largely based on experimental models, studies on focal epileptogenesis have taken advantage of intracranial recordings in the setting of neurosurgery for epilepsy patients. These studies have contributed to shape the now widely accepted concept that clinical seizures in focal epilepsies originate in the ‘seizure-onset zone’ (SOZ), while epileptic seizure activity is generated in the epileptogenic zone (EZ), i.e. the cortical area which is indispensable for seizure generation independent of their clinical manifestation (83). Additional specific cortical areas that can be identified in the epileptic brain are the irritative zone (IZ), representing the area of the cortex generating interictal spikes, the epileptogenic lesion (EL), which may correspond to either a macroscopic epileptogenic lesion (e.g., focal cortical dysplasia) or the hyperexcitable adjacent cortex, and the functional deficit zone (FDZ), representing the area of cortex that does not function normally in the interictal period (84) (FIGURE 2B). The EZ may be either more or less extensive than the SOZ. If the EZ is smaller than the SOZ, even its partial resection or disconnection may result in seizure disappearance, as the remaining SOZ could not be sufficient to generate further epileptic seizures. Conversely, if the EZ is larger than the SOZ, total removal of the SOZ cannot ensure seizure disappearance because multiple SOZs with different thresholds
may coexist in the same EZ. Indeed, in this case, after the resection of the first SOZ, another SOZ with higher threshold may become clinically evident (83). However, this picture is simplistic. Although seizures tend to have preferential spreading patterns, cortical connections spread in all directions, from any given cortical point, through cortico-cortical and subcortical connections (FIGURE 2A, B). Consequently, disconnection of specific networks does not guarantee that seizure activity does not progress via alternative pathways, resulting in a modified clinical semiology, but not in their disappearance (84). Only the complete disconnection or removal of all the potential SOZs can guarantee that seizures disappear.

Some of the seizure types observed in epileptic encephalopathies are almost exclusively seen within this category of severe epilepsies and do not fall within the categorization of generalized or focal epileptogenesis. Epileptic spasms and tonic seizures, which are some of the signature seizures in ISS and LGS, are considered to be bilateral seizures. The possible involvement of the brainstem in generating these seizures was demonstrated in 1958 by Kreindler and collaborators who reported bilateral tonic convulsions in cats and rats when stimulating the reticular substance and periaqueductal substance (85). These certainly may explain reports of epileptic spasms in infants with hydranencephaly (86), although they do not certainly exclude the contribution of other higher structures which could activate a broader network generating these tonic seizures.

Animal models of ISS for example, have provided evidence that cortical or cortico-hippocampal lesions may suffice to trigger spasms (87, 88). Seizures and epileptic activity may also be multifocal, as in Epilepsy of Infancy with Migrating Focal Seizures (EIMFS), as an expression of a widespread, genetically determined epileptogenesis in immature brains with incomplete myelination and poorly functioning connections (89).

III. MAIN (KNOWN) DETERMINANTS OF NORMAL AND ABNORMAL BRAIN DEVELOPMENT THAT INFLUENCE EPILEPTOGENESIS
Normal brain development is a dynamic process that proceeds asynchronously and at different tempos and trajectories across brain regions, cell types and sexes, and is further modified by biological or environmental factors (FIGURE 3). This asynchronous and timed maturation also extends to key developmental processes that potentially control the susceptibility to seizures and epileptogenesis: migration and differentiation of progenitors of excitatory and inhibitory neurons, neurogenesis and synaptogenesis of excitatory and inhibitory synapses, morphological changes of various brain regions (FIGURE 3A), signaling modes of molecular or electrophysiological signaling cascades that control neuronal excitability, differentiation, function or communication, and survival (FIGURE 3B) (90).

It is generally considered that around postnatal day 10 (PN10), a rodent is equivalent to a full term human neonate, an assumption based on studies of brain growth spurt (91). Brain growth spurt in these studies included gross brain growth, DNA, cholesterol, and water content. Puberty onset occurs around PN32-36 in female rats and PN35-45 in male rats, whereas in humans it starts around 10-11 years in girls and 11-12 years in boys (92). Distinct processes, such as neurogenesis and migration, synaptogenesis and synaptic pruning, myelination follow different time courses (FIGURE 3B) (90, 93–100). For studies that specifically target or relate to specific developmental processes it is therefore important to consider the maturational trajectories of the specific developmental processes of interest across species, when these are known.

There are extensive and continuous molecular, morphological or functional changes through development that significantly diversify the effects of epileptogenic processes in the brain, thus rendering the immature brain more amenable to developing the intense and sometimes multifocal epileptic activity associated with DEEs (90). Ontogenetic studies with kindling, a method of repetitive electrical stimulations of limbic structures that results in a progressively more severe seizure phenotype, demonstrated that post-ictal refractoriness to manifest another seizure is
shorter in developing animals, which is likely a factor that predisposes them to develop clusters of seizures (101). In addition, kindling antagonism, whereby kindling stimulation at one limbic structure inhibits the kindling development in another, is not operative in immature pups (102). Although the immature brain is more prone to seizures and seizure clusters than adults, it is also more resilient, demonstrating no or less severe injury after prolonged seizures (103, 104).

The trajectories of maturation of these developmental processes often follow age, sex, and region-specific patterns. These have been extensively studied for GABA\(_A\) receptors (GABA\(_A\)R) and include changes in receptor composition and kinetics of, depolarizing/hyperpolarizing postsynaptic GABA\(_A\) receptor (GABA\(_A\)R) responses or network effects (105–111) (FIGURE 4). GABA\(_A\)R signaling is usually depolarizing in immature neurons with relatively high intracellular Cl\(^-\) content and elicits hyperpolarizing responses in mature neurons which have low intracellular Cl\(^-\) (112–117). The polarity of GABA\(_A\)R responses depends upon the relative abundance of cation chloride cotransporters and channels that import [e.g., NKCC1, abundant early on, decreases later in life (118–120)] or export [e.g. KCC2, expression increases postnatally (121, 122)] Cl\(^-\), in a process that requires energy generated by Na\(^+\)/K\(^+\) ATPase (FIGURE 4). Depolarizing GABA can have neurotrophic effects in immature neurons and is a normal physiological phenomenon needed for normal development (123–125). The developmental shift of GABA\(_A\)R signaling from depolarizing in immature neurons to hyperpolarizing in mature neurons has been proposed to follow a rostro-caudal gradient, with earlier maturation in the most caudal regions. In reality, there are significant cell type-, region-, and sex-specific factors that create a more complex temporospatial pattern of maturation not only of GABA\(_A\)Rs, but also of other neurotransmitter and signaling pathways. For example, hyperpolarizing GABA\(_A\)R responses may occur earlier in rat CA1 pyramidal neurons than in substantia nigra pars reticulata GABAergic neurons (111, 126); GABA\(_A\)R mediated hyperpolarizing responses are already seen in neonatal thalamic neurons, in contrast to cortical
neurons which mature later on (127). The maturation to hyperpolarizing GABAAR signaling may occur earlier in females than in males in certain brain regions (hippocampus, substantia nigra) (108, 111, 125, 126, 128), whereas it may emerge later in females in others (cerebellar Purkinje cells) (129). Premature cessation of depolarizing GABAAR signaling may disrupt the excitatory synapse formation and dendritic arborization of cortical neurons, leading to neurodevelopmental deficits (130–132). Disruptions of the GABAAR-sensitive patterns of communication across cells or brain regions may also occur with seizures or epileptogenesis, stressors, genetic variants, drugs or metabolic disorders, altering threshold for ictogenesis or epileptogenesis and predisposing to behavioral or cognitive impairments (109, 125, 126, 133). Depolarizing GABA can also be seen under pathological conditions, e.g. after axonal injury, hypoxia/hypoglycemia, during prolonged seizures, or in epileptic tissue and its significance there can be dual: partly protective to promote neuronal healing, but also potentially epileptogenic as it may promote neuronal excitability and epileptogenesis (133). Further, KCC2 effects are not strictly upon Cl⁻ regulation; the C-terminus of KCC2 may promote synapse formation (134) and the N-terminus can affect neuroprotection (135, 136). Loss of function mutations in KCC2 have been found in epilepsy syndromes, including Epilepsy of Infancy with Migrating Focal Seizures (EIMFS) (137–139).

GABA signaling is also an important regulator of cortical-subcortical networks that control fundamental physiological functions, such as learning and memory, but also seizures (110, 140–142). The modus operandi of these networks undergoes significant age- and sex-dependent changes through development. The substantia nigra pars reticulata (SNR), largely composed of GABAergic interneurons, acts as a gate through which the cortico-striatal input may activate or inhibit the activity of thalamo-cortical neurons. In adults, activation of GABAARs in the SNR exerts anticonvulsant effects in a variety of seizure models (143–145). However, in developing rodents, the outcome of GABAAR-sensitive SNR-mediated seizure control (anticonvulsant vs proconvulsant
Excitatory neurotransmitters also undergo significant developmental changes in expression and subunit composition (FIGURE 3), which modify their biological effects in regard to both seizure susceptibility and control (110, 146) and neuronal survival (147).

An important concept in developmental epileptogenesis is the existence of critical or sensitive periods for the development of specific traits. The effects of biological factors or exogenous insults can be time-locked to specific developmental periods that render the brain sensitive, as shown for the hormonal regulation of the differentiation of seizure-controlling subcortical networks (148).

The biological roles of channels may also be age-dependent, as demonstrated for example for M channels (149). M channel activity is essential for the normal morphological development of hippocampus but only during the first postnatal weeks of murine development. Loss of M channel activity at later periods does not have overt morphological sequelae in the hippocampus, although cognitive deficits as well as increased neuronal excitability can still be observed. The therapeutic effects of a treatment can be specific for certain developmental periods when they can modify their desired targets, as shown for neonatal estradiol given to prevent interneuronopathy in an Arx knock-in mouse model of epileptic spasms (150). The effects of genetic mutations may differ depending upon the developmental period when these are expressed, as shown for the GABA_α Ry2(R43Q) epilepsy mutation (151).

Beyond gene effects, critical developmental periods are also important in determining the severity of dysfunction conferred by the abnormal excitability. Using bicuculline and penicillin application in the visual cortex of rabbits to induce sporadic epileptiform discharges, Chow and colleagues had shown that the ensuing epileptic activity disrupted the appearance of complex and oriented–directional type cells at the lateral geniculate nucleus in developing, but not in adult, rabbits (152).
More recent studies also demonstrated that the induction of epileptiform discharges by electrical stimulation of the hippocampus in developing animals disrupts place cell formation (153, 154).

IV. NON-GENETIC DETERMINANTS OF THE EPILEPTOGENIC RISK

Inflammation and cytotoxic injury may trigger a chronic and evolving ISS phenotype in the multiple hit model, suggesting that the cortical-subcortical network disruption due to the structural lesion, in tandem with neuroinflammation, may trigger spasms and epileptogenesis with neurodevelopmental deficits (88, 155–161). In the same model, dysregulation of the mTOR (mechanistic target of rapamycin) pathway was a critical pathogenic feature and restoration of its activity with rapamycin resulted in partial improvement of the cognitive deficits and reduced epilepsy development, lending further support for the central role of mTOR in epileptogenesis (155, 161). This model also provides evidence for sleep-epilepsy interplay with most adult motor seizures emerging from sleep, as well as evolution to a slow spike-wave EEG, reminiscent of Lennox-Gastaut syndrome (LGS) in adulthood (155).

The deficit in parvalbumin (PV) positive interneurons from the contralateral cortex (159) is reminiscent of the interneuronopathy seen in ARX-related ISS (150, 162, 163), although the quality of the interneuronopathy under these conditions is different. Mechanistically, these also seem different, since the neonatal estradiol treatment that corrected the ARX-related interneuronopathy and epilepsy (150, 163) did not improve the phenotype of the multiple-hit rats (157).

Stress has been long advocated as a key pathogenic mechanism of ISS (164, 165). Administration of corticotrophin releasing hormone (CRH) in the cortex or hippocampus of pups induced seizures, but not spasms (166). Exposure to conditions that mimic aspects of early life stressors or stress response, such as prenatal betamethasone or stress (167–169), or that disrupt adrenal function aggravated NMDA induced spasms (170), although some of these conditions (such as prenatal
betamethasone) enhanced responsiveness of NMDA-induced spasms to ACTH (168). A more recent model of chronic early life stress due to fragmented nurturing behaviors was reported to manifest a mild spasms phenotype (171).

V. GENETIC DETERMINANTS OF DEE: EXPERIMENTAL MODELS

DEEs causing genes can be grouped into broad functional categories involved in different cellular processes including ions/transmitters/small molecules transport, regulation of synaptic function, cell growth, division and proliferation, cell metabolism, intracellular trafficking and signaling, gene transcription, and protein biosynthesis/degradation. (TABLE 2 and SUPPLEMENTAL TABLE S1, https://figshare.com/articles/dataset/Guerrini_et_al_Supplemental_Table_S1/19666521).

We will focus upon gene products which have been extensively studied for their effects on neuronal excitability and epileptogenesis in *in vitro* and *in vivo* studies, using relevant natural or genetically engineered models. They offer clearer targets to design rational therapies to restore normal function in dysfunctional networks which can be easily assessed using physiological tests. We will also discuss more succinctly other genes that emerged as genes of interest for certain DEEs, particularly those associated with migration disorders; however, their mechanisms of action are more complex, involving multiple cellular processes that need to be disentangled before designing safe and effective therapies.

Neuronal circuits are formed by principal glutamatergic excitatory neurons and inhibitory GABAergic neurons (FIGURE 5). It is thought that in cortical circuits glutamatergic neurons perform computational tasks, whereas GABAergic neurons control and organize the activity of the network and are important for the generation of rhythms of activities, which are the substrate of brain rhythms. Glial cells are important not only for neuron homeostasis and protection but are implicated in synaptic functions. In neurons, the somato-dendritic compartment receives most of the synaptic inputs that are computed in the dendritic tree and integrated in the axon initial
segment, where APs are generated. Forward-propagating APs reach presynaptic terminals, where they evoke neurotransmitter release. Back-propagating APs in the somato-dendritic compartment are implicated in dendritic computation and modulation of synaptic inputs. Ion channels are essential in all neuronal sub-compartments and for neuronal signaling. Thus, it is not surprising that channelopathies are implicated in numerous DEEs (172) (FIGURE 5).

Functional analysis of mutations using a variety of experimental systems is essential for shedding light on the detailed pathomechanisms and clarifying genotype-phenotype correlations, which can in turn facilitate diagnosis, genetic counselling, management, and development of therapeutic approaches. Functional analysis using electrophysiological techniques identifies even subtle modifications in the properties of ion channels. Experimental methods include both in vitro and in vivo systems (173, 174), (FIGURE 6).

In vitro experimental systems often use cells that do not endogenously express the protein of interest, thus simplifying the functional analysis of its properties. They are in general human cell lines (e.g., transfected human embryonic kidney, HEK, cells) or, less frequently, oocytes of the clawed frog *Xenopus laevis* injected with the cRNA of interest that allow massive expression, although a human cellular background is generally preferable. Transfected/transduced neurons in primary cultures are a further in vitro system that provides a true neuronal cellular background to evaluate effects on neuronal and network properties. In vivo/ex vivo systems are organisms or preparations obtained from them (e.g., brain slices), which should better model the complexity of brain circuits and the actual pathophysiological conditions, as well as provide information of in vivo phenotypes. The animals more frequently used for generating in vivo models of genetic variants are mice and rats (TABLE 3), because early site directed mutagenesis techniques exploiting homologous recombination in embryonic stem cells allows easy genetic manipulation of these mammals. The mouse is still the organism of choice for generating animal models of genetic
variants, although more recent methods of genome editing (e.g., CRISPR-Cas9) can be used to
generate mutant models with other mammals. It is not possible to perform high throughput
studies with mammalian models, neither for studying functional effects of variants nor for drug
screens. Simpler animal models make it possible to perform relatively large screens, in particular
the zebrafish, which has vertebrate features (175). However, findings obtained with these simple
systems need to be validated in mammalian models. Neurons differentiated from induced
pluripotent stem cells (iPSCs) generated from patients' biopsies are increasingly used to study
mutations in human neurons as they bear the patient’s genetic background. They can be used for
investigations of neuronal properties at the single cell level or can be induced to generate in vitro
miniature organs resembling the brain (brain organoids) that represent an excellent integrated
experimental system to study brain development (176). However, large variability in the
properties of these neurons makes studies difficult and reproducibility is still an issue, as observed
in studies of SCN1A mutations (177–184).

VI. DYSFUNCTIONS IN NEURAL CELL MIGRATION, PROLIFERATION, AND SYNAPTOGENESIS

Mutations in distinct DEE genes can trigger different molecular and biochemical alterations which,
depending on the developmental stage involved and the type of alteration, may result in a brain
with a grossly abnormal morphology or structurally normal, but functionally abnormal (FIGURE
7A-BB). Disruption of any of the overlapping steps that contribute to the development of the
human cerebral cortex that can be recognized as malformations in studies of brain imaging is
designated as “malformation of cortical development” (MCD). MCDs can be broadly classified into
three major groups that recapitulate the main developmental steps, i.e., malformations of cell
proliferation, neuronal migration, or post-migrational cortical organization (185).

Among brain morphological abnormalities, MCDs are those most frequently associated with
recurrent seizures. In MCDs, altered processes of development involve cells that, under normal
circumstances, would participate to the formation of the normal cerebral cortex, and seizures can arise because of neuronal malpositioning, abnormal proliferation or differentiation, or abnormal cortical organization (186).

Neuronal malpositioning is the result of altered neuronal migration during brain development. A classic example of DEE associated with neuronal migration deficit is caused by mutations in the aristaless-related homeobox (ARX) gene, located on chromosome Xp21, which can result in a phenotypic spectrum comprising a nearly continuous series of neurodevelopmental disorders including lissencephaly with ambiguous genitalia (XLAG) (FIGURE 7A), Proud syndrome, Partington syndrome, infantile spasms without brain malformations, and syndromic and non-syndromic intellectual disability with epilepsy.

The ARX protein belongs to the Aristaless-related subset of the paired (Prd) class of homeodomain proteins. Homeodomain transcription factors play crucial roles in cerebral development and patterning (187). In particular, ARX is involved in the normal tangential migration of GABAergic neurons and the occurrence of seizures in most patients carrying mutations in this gene can be ascribed to mislocalization or malfunction of this class of neurons and loss of inhibitory neurotransmission (188). For this reason, ARX-related DEEs is considered a “developmental interneuronopathy”, a term coined to differentiate developmental brain disorders caused by impaired development, migration, or function of interneurons from functional deficits or secondary loss of interneurons and from the more common channelopathies (189).

Developmental interneuronopathies may also include Dravet syndrome, as discussed above, and classical lissencephaly due to mutations in PAFAH1B1, also known as LIS1 (FIGURE 7B, C), or DCX (FIGURE 7D). The PAFAH1B1 gene encodes for the regulatory beta-subunit of the cytosolic type I platelet-activating factor (PAF) acetylhydrolase, which is involved in interneuronal migration and survival, whilst DCX encodes for a microtubule-associated protein essential for both radial and
non-radial interneuronal migration into the cerebral cortex (55). Interneuronopathies have also
been described in models of acquired DEEs, such as the multiple hit model of ISS (159), although
the underlying interneuronal deficits and mechanisms are likely distinct, requiring different
targeted therapeutic approaches (157).

The phenotypic continuum of MCDs, which includes focal cortical dysplasia type II (FCDII),
hemimegalencephaly (HME), megalencephaly (MEG), and dysplastic megalencephaly (DMEG), is
mainly caused by constitutional and somatic mutations in mTOR pathway genes, i.e., AKT1, AKT3
(FIGURE 7E), DEPDC5, MTOR (FIGURE 7F, G), NPRL2 (FIGURE 7H), NPRL3, PIK3CA, PIK3R2 (FIGURE
7I, J), PTEN (FIGURE 7K, L), TSC1, and TSC2 (FIGURE 7M, N), and represents the paradigm of DEEs
caused by abnormal neuronal proliferation or differentiation, in addition to abnormal neuronal
migration. FCD is the cause of seizures in almost half of children referred for surgical treatment of
medically refractory epilepsy, roughly estimated to near 400,000 people in the United States
(Wolters Kluwer UpToDate website - https://www.uptodate.com/contents/evaluation-and-
management-of-drug-resistant-epilepsy). In addition to abnormal cortical lamination, FCDII also
features large dysmorphic neurons without (type IIa) or with (type IIb) balloon cells (190). HME, a
condition in which one hemisphere is abnormally larger than the contralateral, and DMEG, a
condition in which cortical dysplasia is associated with segmental brain overgrowth, exhibit
histopathological features similar to FCDII (185). Tuberous sclerosis complex (TSC), a congenital
syndrome characterized by the development of benign tumors (hamartomas) in multiple organs,
including the brain, and a neurological phenotype consisting mainly of early-onset seizures,
intellectual disability, and at times, autism, is also caused by mTOR pathway dysregulation due to
mutations in the tumor suppressor genes TSC1 or TSC2 (191).

Balloon cells in FCDIIb and TSC exhibit features of both neurons and glia. On histopathological
analysis, dysmorphic or cytomegalic neurons are often associated with increased proliferation of
normal-appearing glia or abnormal reactive astrocytes, which overexpress specific intermediate filaments and other immature molecular markers. Taken together, these findings strongly implicate primary defects in cellular proliferation and differentiation in the pathogenesis of mTORopathies (192).

Electrophysiological recordings in neocortical samples surgically removed from patients with FCD have demonstrated that, under reduced K⁺ conductance, dysmorphic cytomegalic neurons, but not balloon cells, generate large Ca²⁺ currents when stimulated, indicating that this aberrant cell type plays an important role in epileptogenesis (193). Both the morphology and size of dysmorphic cytomegalic neurons have been implicated with the origin of aberrant electrical discharges. By using electrophysiology, calcium imaging, morphological analyses, and modeling studies, Williams and collaborators demonstrated that Pten (an mTOR pathway inhibitor) knock-out neurons exhibit rapid-onset hypertrophy and higher density of synapses proximal to the soma and that these phenotypic abnormalities promote firing at more hyperpolarized membrane potentials, with greater peak spike rates, and higher sensitivity to depolarizing synaptic input (194).

Using mouse models with cell-type-specific mTOR pathway activation obtained by electroporating the Pik3ca p.H1047R mutation, which is associated with both FCD and HME, D’Gama and collaborators demonstrated that mTOR pathway activation in excitatory neurons and glia, but not in interneurons, is sufficient to cause abnormal cortical lamination and overgrowth (195). In FCD, epileptic seizures appear to be triggered by a peculiar mechanism which we detail below. By treating in vitro-maintained organotypic slice cultures of resected FCD lesions with 4-aminopyridine, a potent convulsant agent acting as potassium channel blocker, Avoli and collaborators demonstrated intrinsically generated ictal-like epileptiform events (196). These epileptiform events were triggered by an amino acid receptor-mediated mechanism which was glutamatergic-independent and mainly attributable to a GABA_A receptor-mediated conductance...
29. These findings are in line with the observation that, in some cases, dysplastic tissue of FCDIIb and cortical tubers retain features of immature tissue (197, 198).

VII. DYSFUNCTIONS IN INTRINSIC EXCITABILITY

1. VOLTAGE-GATED Na\(^+\) CHANNELS

Na\(^+\) channels are clustered at high density at the axonal initial segment (AIS), which is, for this reason, the primary site for generation of APs in neurons (FIGURE 5). In myelinated axons, Na\(^+\) channels are clustered at high density also at the nodes of Ranvier to allow saltatory axonal conduction. Voltage-gated Na\(^+\) channels are essential for the generation of neuronal excitability because they generate the depolarizing Na\(^+\) current that initiate and propagate APs. These Na\(^+\) currents have fast activation and inactivate within few milliseconds after opening, although a small fraction of slowly inactivating (persistent) current remains for longer periods during depolarizations (199, 200). Nav are composed by a principal pore-forming α subunit (nine isoforms: Na\(_{\alpha}1.1\)-Na\(_{\alpha}1.9\) for the proteins, SCN1A-SCN11A for the genes) and auxiliary β subunits (four isoforms: β1-β4 for the proteins, SCN1B-SCN4B for the genes) (199, 201). The primary sequence of the α subunits contains four homologous domains (D1-DIV), each comprising six predicted transmembrane segments (S1-S6) that form voltage-sensing modules (S1-S4; S4 is the voltage sensor) and pore modules (S5-S6 and their connecting extracellular loop) in each domain. The β subunits contain a single transmembrane segment. SCN5s/Na\(_{\alpha}s\) are targets of clinically used sodium channel blockers, among them several anti-seizure medications (ASMs) (202). Mutations in SCN1A/Na\(_{\alpha}1.1\), SCN2A/Na\(_{\alpha}1.1\), SCN3A/Na\(_{\alpha}1.1\) and SCN8A/Na\(_{\alpha}1.6\) as well as of SCN1B/β1, which are expressed in the central nervous system, are important causes of DEEs (199, 203–205). Patients carrying variants in voltage gated Na\(^+\) channels also exhibit an increased risk of sudden unexpected death in epilepsy (SUDEP) (206). Mutations implicated in DEEs have been found also in genes encoding proteins that can modulate Nav properties, like FGF12/FHF1 (207, 208).
1.1. Na\textsubscript{v}1.1 channels (SCN1A)

SCN1A, encoding the Na\textsubscript{v}1.1 α subunit, is one of the most clinically relevant epilepsy genes, with hundreds of mutations reported thus far, whose associated phenotypes range from Dravet syndrome, a severe form of DEE, to milder GEFS+ and other diseases, such as hemiplegic migraine (4, 209, 210).

Dravet syndrome is caused by often \textit{de novo} heterozygous SCN1A mutations (211, 212), of which approximately half are missense, and half are predicted to give rise to a truncated non-functional protein. The clinical spectrum of Dravet syndrome does not have firmly established boundaries, but the core phenotype is characterized by intractable seizures precipitated by increased body temperature with onset between six months and one year of age, and subsequent appearance of multiple hyperthermia-induced and hyperthermia-independent seizures. Development is normal in the first year of life but plateaus rapidly, with most patients showing cognitive impairment. In patients with Dravet syndrome, SCN1A mutations can also be inherited from a parent with less severe clinical manifestations, at times carrying somatic mosaicism (213). It has been proposed that mutations in other genes can cause Dravet syndrome-like phenotypes (including SCN1B, HCN1, KCN2A, GABRA1, GABRG2, and STXBP1), but with a specific clinical pictures (see below).

Thus, the association between SCN1A mutations and Dravet syndrome is highly specific. The familial epilepsy GEFS+ syndrome can be also caused by heterozygous missense SCN1A mutations and is characterized by febrile seizures plus (FS+: febrile/hyperthermic seizures that extend beyond six years of age) and afebrile generalized tonic-clonic seizures (GTCS), at times including absence, myoclonic, atonic, or focal seizures, and Dravet syndrome. Missense SCN1A mutations can also cause sporadic/familial hemiplegic migraine type3 (S/FHM3), a rare form of migraine with aura and onset in adolescence, characterized by hemiparesis as part of the aura phase. Moreover, two missense mutations with gain of function have been associated with an extremely severe
early infantile DEE with earlier seizure onset, profound cognitive and motor impairments, and a hyperkinetic movement disorder (214–216).

The functional effect of truncating mutations causing Dravet syndrome has since their identification thought to be haploinsufficiency: 50% reduction of functional Na$_v$1.1 protein in heterozygotes, with complete loss-of-function (LoF) (211), which has been verified in functional studies (217). The effect of missense mutations studied in transfected cell lines has been more controversial, but most of the results point to a LoF, whose severity tends to correlate with that of the phenotype (199, 218). Thus, the severity spectrum of SCN1A-related epilepsies could be a continuum and depend on the amount of LoF of the mutant. Some SCN1A missense mutations cause LoF because of folding/trafficking defects that lead to channel degradation (219) that can at least partially be rescued by interacting proteins stabilizing the correct folding conformation (220–223).

Na$_v$1.1 is the predominant Na$^+$ channel of GABAergic interneurons whose decreased excitability induced by SCN1A epileptogenic mutations reduces GABAergic inhibition and causes network hyperexcitability (64). A study with a knock-in model expressing a truncating nonsense DS mutation reported a similar phenotype, showing that Na$_v$1.1 localizes to the axon initial segment of GABAergic interneurons, in particular fast-spiking parvalbumin (PV)–positive ones (65). Subsequent studies have shown that these mice display also co-morbidities including cognitive and behavioral deficits, ataxia, SUDEP, dysregulated circadian rhythms and sleep dysfunctions (199, 218, 224, 225). Several other studies, including those performed with conditional mouse models that expresses the mutations in specific neuronal subtypes (199, 218) and with a knock-in model of a GEFS+ mutation (Scn1a$^{R1648H/+}$) (226, 227) have confirmed that hypoexcitability of GABAergic neurons is the initial pathological mechanism in Dravet syndrome models and that the amount of LoF can determine the severity of the phenotype. Notably, Scn1a mouse models have
also allowed to disclose genetic modifiers and cellular remodeling induced by the initial effect of the \textit{Scn1a} mutation, which can modulate the phenotype implementing homeostatic or pro-pathologic modifications, including homeostatic upregulation of Na\(^+\) channels in GABAergic neurons and seizure-induced hyperexcitability of glutamatergic neurons (199, 218). For instance, the interaction between seizures and the genetic mutation can induce remodeling in the \textit{Scn1a}^{R1648H/+} mouse model transforming a basically asymptomatic phenotype into a Dravet syndrome-like one, because of increased excitability of excitatory glutamatergic neurons (228).

Overall, these results show that the initial pathological mechanism in mouse models of human epileptogenic \textit{SCN1A} mutations is LoF and hypoexcitability of at least some subtypes of GABAergic neurons. However, this initial dysfunction triggers both homeostatic and pathologic remodeling, depending on the neuronal types, age, genetic background, and interactions between \textit{SCN1A} mutations and experienced seizures.

Although some drugs like stiripentol (229), fenfluramine (230) or cannabidiol (231) have been shown to be partially effective in some patients, Dravet syndrome still remains drug-resistant for most of the patients. \textit{Scn1a} gene targeted mice have been used for testing therapeutic approaches, both classical pharmacological treatments and novel methods, including gene therapy, in some cases obtaining significant amelioration of the phenotype (199, 232). Some novel approaches have shown particularly effective results and have led to clinical trials. For example, an antisense oligonucleotide-based targeted augmentation of nuclear gene output (TANGO) approach has been used to increase the expression of functional Na\(_{\text{v}}\)1.1 channels in Dravet syndrome mice, observing a substantial decrease in spontaneous seizures and SUDEP when specific antisense oligonucleotides were injected in newborn mice (233).

Mutations causing sporadic/familial hemiplegic migraine (S/FHM) cause Na\(_{\text{v}}\)1.1 gain-of-function (GoF) by inducing gating modifications and increasing the slowly inactivating “persistent” Na\(^+\)
current (199, 218). Gene targeted mouse models of S/FHM mutations have been generated and they show facilitated/spontaneous generation of cortical spreading depression (CSD), a proposed pathological mechanism of migraine with aura, but no seizures (234, 235). Genetic and acute models have shown that these mutations induce hyperexcitability of GABAergic neurons leading to increase of extracellular $K^+$ and clamped depolarizing block of neuronal activity (236). A very mild GoF has also been observed for the p.T226M mutation causing an extremely severe early infantile $SCN1A$ DEE (216), but it is not clear yet how such a mild change could induce the severe phenotype.

1.2. Na\(\text{v}1.2\) channels ($SCN2A$)

$SCN2A$ encodes the Na\(\text{v}1.2\) sodium channel, which is widely expressed in the central nervous system, particularly in cortical and hippocampal glutamatergic neurons (199). These are the main Na\(^+\) channels of the axonal initial segment (AIS) and the nodes of Ranvier in the first 10 days of postnatal life in rodents, then they are partially replaced by $SCN8A$/Na\(\text{v}1.6\). It is thought that Na\(\text{v}1.2\) channels in the proximal AIS promote backpropagation to the soma. In the adult brain, Na\(\text{v}1.2\) is also present in thin processes, presumably distal unmyelinated portions of preterminal axons.

The first epilepsy syndrome clearly associated with $SCN2A$ mutations was benign familial neonatal/infantile seizures (BFNIS) (237), characterized by a mild phenotype. Subsequently, it has been shown that mutations in $SCN2A$ cause a wide range of neurodevelopmental disorders, including DEE of varying severity. DEE mutations generally arise de novo, and about 80% are missense. About 60% of $SCN2A$ DEE have onset in the first three months of life (238, 239). All neonatal-early infantile DEE patients display intellectual disability. About 50% of them have variable seizure types, whereas the others have Ohtahara syndrome (neonatal-onset spasms or tonic seizures and EEG with burst suppression pattern), sometimes evolving into West syndrome,
Lennox–Gastaut phenotypes or epilepsy with early infantile migrating focal seizures (EIMFS). DEE with infantile and childhood onset are about 40% of the total (238, 239). The phenotype can be correlated to the age of onset. Patients with onset between three months and one year of age in general have West syndrome, with possible evolution into a Lennox–Gastaut phenotype. Patients with onset after one year of age often show variable seizure phenotypes with developmental/cognitive delay and autistic traits that can appear before seizure onset.

Functional analysis in transfected cell lines has shown some genotype-phenotype correlations (199, 240). GoF mutations are related to mild benign neonatal/infantile epilepsy and to neonatal/early infantile DEE, which can be responsive to treatment with Na⁺ channel blockers (239), whereas LoF mutations are linked to infantile/childhood epileptic encephalopathy or neurodevelopmental disorders (autism and intellectual disability) without seizures. However, identifying genotype-phenotype correlations within these GoF and LoF mutants is difficult due to partially overlapping phenotypes.

Recordings in brain slices of heterozygous knock-out Scn2a (Scn2a⁺/-) mice have shown that Naᵥ1.2, besides its established axonal role, has important dendritic functions in pyramidal neurons of the prefrontal cortex, where its haploinsufficiency impairs synaptic plasticity and synaptic strength, even when Scn2a expression is reduced in single neurons late in postnatal development (241). However, these studies have shown that Scn2a⁺/- mice, differently than Scn1a models, have a relatively mild phenotype, including short absence-like seizures, spatial memory deficits but enhanced fear memory, plus autistic traits (199, 242). A Scn2a mouse model carrying the severe hypomorphic mutation Δ1898 shows a more severe phenotype, with robust autistic-like features (243). Two additional studies have shown that either complete deletion (244) or reduced expression of Scn2a (244, 245) can paradoxically induce hyperexcitability of glutamatergic neurons. Overall, these findings suggest that reduction of SCN2A to more than haploinsufficiency
could be needed to induce severe phenotypes. A knock-in mouse model of the recurrent GOF mutation R1882Q has been generated (246) showing that heterozygous mice display hyperexcitability of cortical pyramidal neurons, and develop spontaneous seizures at P1 and premature death between P13 and P30. Interestingly, reduction of Na\textsubscript{v}1.2 expression by specific antisense oligonucleotides reduced seizures and extended lifespan.

1.3. Na\textsubscript{v}1.3 channels (**SCN3A**)

**SCN3A** encodes the Na\textsubscript{v}1.3 Na\textsuperscript{+} channel, whose functions have not been completely determined yet. **SCN3A** is widely expressed in the brain at high levels during embryonic development and is downregulated afterwards (199).

**SCN3A**-related clinical phenotypes comprise a wide spectrum, including mild epilepsy with intellectual dysfunction, early infantile DEE often associated with polymicrogyria, as well as speech and oral motor dysfunctions associated with polymicrogyria without epilepsy. Relatively mild neonatal-childhood onset focal epilepsy (247), would be the mildest phenotype in the spectrum (248, 249). Disrupted cerebral cortical folding and neuronal migration were recapitulated in ferrets expressing the mutant **SCN3A** (249). Functional studies in transfected human cell lines have shown that most of the **SCN3A** mutations associated with severe human phenotypes exhibit prominent GoF, inducing, in particular, large increases of persistent Na\textsuperscript{+} current (248–250). LoF caused by reduced current density has been reported for some **SCN3A** variants. Heterozygous adult Scn3a\textsuperscript{+/-} knock out mice were investigated as a model of **SCN3A** LoF mutations, but they do not show features of DEE (251).

1.4. Na\textsubscript{v}1.6 channels (**SCN8A**)

**SCN8A** encodes Na\textsubscript{v}1.6, the main Na\textsuperscript{+} channel in the axon initial segment and nodes of Ranvier of myelinated axons in mature excitatory neurons (199).
De novo heterozygous mutations in SCN8A have been identified in a range of phenotypes. Most patients show a DEE with multiple seizure types and early onset, severe intellectual disability and movement disorders and have an increased risk of SUDEP (252). Other patients exhibit milder phenotypes, including benign familial infantile seizures (BFIS) and epilepsies with intermediate phenotypes, or generalized epilepsy with absence seizures (253, 254). Some patients show intellectual disability, autism, or movement disorders without epilepsy (255, 256). About 20% of patients have recurrent mutations.

Mutations that cause DEE or milder epilepsy induce GoF with negative shifts of voltage-dependence of activation, positive shifts of voltage-dependence of inactivation, slowed channel inactivation, or increased persistent or resurgent current. These functional changes are all consistent with neuronal hyperexcitability, which has been confirmed in transfected cultured neurons (255, 257). Other mutations causing intellectual disability, autism, or movement disorders without epilepsy induce LoF, which can be complete (255, 256). Massive GoF can induce reduced generation of APs, mimicking LoF mutations (255). LoF mutations have been identified in patients with absence seizures (254), a phenotype reproduced by heterozygous loss of function mouse models.

A standard global knock-in (258) and a conditional floxed knock-in (259) mouse models of SCN8A DEE mutations are available. Overall, knock-in mice indicate that SCN8A GoF mutations are sufficient to induce hyperexcitability of some subtypes of excitatory neurons, generating severe seizures and a lethal phenotype. In contrast, spontaneous mouse models carrying LoF SCN8A mutations (260) show a phenotype similar to that of patients with intellectual disability and/or movement disorders without epilepsy.

Some patients with SCN8A GoF mutations respond to high doses of sodium channel blockers (254, 261), but available blockers are not isoform-specific and chronic therapy at high doses can induce
adverse effects. A recent study has used antisense oligonucleotides that reduce Scn8a expression by 25–50%, showing a delay of seizure onset and lethality in a knock-in Scn8a mouse model (262). These genetic approaches are highly specific for SCN8A, but problems of oligonucleotide delivery and half-life need to be solved before a translational use.

1.5 Auxiliary subunits of Na⁺ channels

SCN1B encodes the “auxiliary” β1 Na⁺ channel subunit that is widely expressed in different organs, including the central nervous system. β-subunits were originally named auxiliary because they do not directly form the channel but modulate the properties and the membrane delivery of α-subunits. We now know that they are multifunctional molecules implicated, besides direct modulation of α subunits, in diverse and essential roles in multiple tissues, including cell adhesion and migration, neuronal pathfinding, fasciculation and neurite outgrowth (204). Mutations in β-subunits can alter numerous functions, including modulations of all the α-subunits, and thereby they are involved in epilepsy, neurodegenerative disorders, neuropathic pain, cardiac arrhythmias and cancer (204). Mutations in SCN1B have been identified in a range of epilepsy phenotypes. Homozygous SCN1B mutations have been identified in DEE patients that were initially included in the Dravet syndrome spectrum (263). However, it is now clear that their clinical features are distinct from Dravet syndrome, showing earlier onset seizures and more severe neurodevelopmental phenotype, including psychomotor, stagnation or regression, and microcephaly (264, 265).

Functional analysis of SCN1B DEE mutations in transfected cell lines have identified either LoF modulation of co-expressed α subunits or induction of complex gating modifications in different co-expressed α subunits (263–265). Homozygous knock-out null Scn1b⁻/⁻ mice show spontaneous seizures and a high mortality rate (266). Cortical neurons in brain slices obtained from these mice have shown dysfunctions in the excitability of both pyramidal excitatory neurons and GABAergic
fast-spiking interneurons. GABAergic interneurons were hypoexcitable, whereas dysfunctions of pyramidal neurons were more complex, with subsets of them exhibiting hyperexcitability at low current injections, as well as hypoexcitability at high stimulation intensities.

2. VOLTAGE-GATED K+ CHANNELS

K+ channels are the most diverse group of ion channels and can be classified into different families depending on the number of transmembrane domains in each subunit and the gating mechanisms. Voltage-gated K+ channels (Kv) are composed by four subunits, each with six transmembrane segments, a voltage sensor module formed by segments S1 to S4, and the pore region formed by S5, S6 and their connecting loop, as in NaV channels. They generate repolarizing currents that oppose the action of depolarizing currents and are involved in different epileptic encephalopathies.

2.1. M-current K+ channels (Kv7, KCNQ)

Five KCNQ genes have been identified that encode Kv7 K+ channels, which can form both homo- and hetero-tetramers (267, 268). Of the four channels expressed in the nervous system (KCNQ2-5), three (particularly KCNQ2, but also KCNQ3 and KCNQ5) host DEE mutations. KCNQ2 and KCNQ3 are expressed in many brain areas, whereas expression of KCNQ5 is more limited (269). They generate the M (muscarinic receptor inhibited)-current, a slow non-inactivating K+ current that activates at subthreshold membrane potentials (270). KCNQ2-linked DEE has its onset in early infancy and features motor impairment and variable intellectual disability (271). Often, the EEG initially shows a burst suppression pattern that may later evolve to multifocal epileptiform activity. KCNQ3 and KCNQ5 DEEs display a variable degree of severity and some patients show intellectual disability apparently without epilepsy (9, 267). Mutations in the three KCNQ genes can also cause “benign familial neonatal seizures” (BFNS), which is mild and self-limited (268).
According to their subcellular localization, $K_V$ channels can regulate specific features of neuronal excitability. Somato-dendritic channels are strongly activated by back-propagating APs, attenuate repetitive firing and contribute to the medium and slow components of the afterhyperpolarization (AHP), determining the refractory period and regulating spiking frequency. In the perisomatic region, slow activation of the M current decreases neuronal firing in response to sustained stimuli, inducing spike-frequency adaptation (269, 272, 273). Axonal $K_V$ channels mostly function by stabilizing the resting membrane potential, leading to increased activation of axonal $Na_V$ channels (274–276) and of presynaptic $Ca^{2+}$ channels, modulating synaptic release (277, 278). Both $KCNQ2$ and $KCNQ3$ are also expressed in GABAergic interneurons (279).

Numerous *in vitro* studies have investigated functional effects of $KCNQ$ DEE mutations in cell lines or *Xenopus* oocytes (267). Most studies agree that LoF, often leading to haploinsufficiency, is the pathophysiologic mechanism of mild and self-limited BFNS (267, 268). DEE mutations can induce more severe LoF consistent with a dominant negative effect. A single report showed that the recurrent p.A294V $K_V7.2$ pore mutation does not modify the properties of the current, but induces LoF altering the subcellular localization of $K_V$ channels (280). DEE can also be caused by GoF, in which case, disease severity correlates with that of functional alteration (267). The first transgenic mouse model expressing a $KCNQ2$ dominant-negative mutant (149), showed spontaneous focal and generalized tonic-clonic seizures, impaired hippocampus-related memory and pronounced hyperactivity, but also cell loss in the hippocampus, which is not observed in patients. The first knock-in model of $KCNQ2$-DEE (281) carrying the p.T274M mutation displayed an overall 70–80% reduction of the M-current (282). $KCNQ2^{T274M/+}$ mice show spontaneous generalized seizures and impairment of spatial learning and memory, and do not show major structural brain defects or neuronal mortality, which is consistent with the clinical features observed in patients. The selective deletion of $KCNQ2$ and $KCNQ3$ in parvalbumin-positive interneurons increased their
firing, leading to homeostatic potentiation of excitatory synaptic activity. Consistently this model shows reduced latency for picrotoxin-induced seizures (283).

### 2.2. Delayed rectifier $K^+$ channels ($K_{v1.1}/KCNA1$; $K_{v1.2}/KCNA2$; $K_{v2.1}/KCNB1$; $K_{v8.2}/KCNV2$)

$K_{v1.1}$ ($KCNA1$) and $K_{v1.2}$ ($KCNA2$). $KCNA2$ encodes the $K_{v1.2}$ shaker-type voltage-gated $K^+$ channel subunit, which is highly expressed in both excitatory and inhibitory neurons, particularly in the axon (284–286). The $K_{v1.2}$ channel generates the delayed rectifier $K^+$ current, an important component of the repolarizing currents during APs. $K_{v1.2}$ channels have high homology to $K_{v1.1}$ channels but require stronger depolarization to activate. $K_{v1.1}$ and $K_{v1.2}$ subunits can produce heteromeric potassium channels with intermediate properties between the respective homomers (287). $K_{v1.1}$ and $K_{v1.2}$ subunits are associated with cell adhesion molecules (CAMs), including LGI1, which contributes to their subcellular localization (288). $KCNA2$-DEE clinical features have been correlated with effects of mutations studied in vitro and can result in strong GoF, mixed GoF and LoF, and LoF with negative dominance (289, 290). Patients with strong GoF variants have onset between five and 15 months of age with intellectual disability, ataxia, and cerebellar atrophy, often with generalized seizures. Patients with variants showing mixed GoF and LoF effects have onset from the neonatal period to six months of age with refractory generalized and focal seizures and developmental delay. Patients with dominant negative LoF variants can have less severe features. The LoF mechanism is consistent with the classical role of $K^+$ channels and the phenotype of $K_{v1.2}$ mutant mice. Homozygous knock-out mice show severe seizures and early mortality, and heterozygous knock-out mice are more sensitive to convulsants, although they do not show spontaneous seizures (291). Both heterozygous and homozygous “Pingu” mice, which carry a chemically induced LoF missense mutation, show motor abnormalities (292). Since $K_{v1.2}$ channels are expressed in both excitatory and inhibitory neurons, the cellular mechanism of $KCNA2$ mutations is not completely clear. Their repolarizing role can help both sustaining high
firing rates and setting the resting membrane potential. Recordings from LoF mouse models have shown contrasting modifications of neuronal firing, with hypoexcitability of glycinergic neurons in \(Kcna2\) knock-out (291) and hyperexcitability of cerebellar basket cells in \(Pingu\) mice (292). Although the detailed pathological mechanism is still elusive, the identification of GoF or LoF effects can provide important information for orienting the therapy in a precision medicine approach. In fact, it has been recently shown that the \(K^+\) channel blocker 4-aminopyridine antagonized GoF defects caused by variants in \(KCNA2\) \textit{in vitro} and was effective in reducing symptoms in patients carrying GoF \(KCNA2\) mutations (293). \(KCNA1\) LoF mutations have also been identified in some DEE patients (294, 295) extending the pathological spectrum of this gene to severe epilepsy.

\(Kv2.1\) (\(KCNB1\)) and \(Kv8.2\) (\(KCNV2\)). \(KCNB1\) encodes the \(Kv2.1\) pore-forming and voltage-sensing \(\alpha\)-subunit, which contributes to generate the delayed-rectifier \(K^+\) current (268). It is expressed in both excitatory and inhibitory neurons of the mammalian brain, and is localized to the soma, proximal dendrites, and axon initial segments (296). \(Kv2.1\) is important for sensing and homeostatically regulating excitability, because its activity can be inhibited by phosphorylation; increased neuronal activity induces dephosphorylation, which results in increased delayed rectifier current leading to reduced neuronal excitability (297, 298). Mutations in \(KCNB1\) have been recently reported in patients with early-onset DEE (299–301). \(KCNB1\)-related DEEs encompass a wide spectrum of neurodevelopmental disorders with different types of epileptic seizures, predominant language difficulties and behavioral impairment. Most variants occur \textit{de novo} and mainly consist of missense variants located on the voltage sensor and the pore domain. Truncating variants in the C-terminal domain are associated with a less-severe epileptic phenotype, although cognitive/behavioral impairment is still severe. Functional studies in transfected cells have shown a variety of defects, including loss of ion selectivity, reduced conductance, and dominant negative
effects, as well as milder effects on gating properties (299, 302, 303), which induce reduced function and increased neuronal excitability. Kcnb1<sup>−/−</sup> mice show hyperexcitable hippocampal circuits but do not show spontaneous seizures, although they exhibit increased seizure susceptibility (298). The voltage-gated K<sup>+</sup> channel subunit Kv8.2, encoded by the KCNV2 gene, is a silent subunit when expressed as a homotetramer, but it increases the Kv2 current when co-assembled as a hetero-tetramer with other Kv2 channels, for example in hippocampal pyramidal neurons, in which it co-localize with Kv2.1 and contributes to the generation of the delayed-rectifier K<sup>+</sup> current (304, 305). De novo LoF variants in the KCNV2 gene, encoding the voltage-gated K<sup>+</sup> channel subunit Kv8.2, have been identified in few DEE patients and contribute to epilepsy susceptibility in mice (305).

2.3. Fast K<sup>+</sup> channels

Kv3.1 (KCNC1) and Kv4.1-4.3 (KCND). KCNC1 encodes the Kv3.1 channel, which is preferentially expressed in neurons that generate high frequency firing, including parvalbumin-containing GABAergic interneurons in the cerebral cortex, hippocampus and amygdala, auditory brainstem neurons, cerebellar granule cells, and neurons of the reticular nucleus of the thalamus (306). In fast spiking GABAergic neurons, Kv3.1 channels are localized to the proximal dendrites, somata, axon hillock, and synaptic terminals, but are not found in distal dendrites (306, 307). Specific functional properties that distinguish Kv3.1 channels from other Kv channels are the very fast kinetics of activation and deactivation, and the voltage dependence of activation shifted towards depolarized potentials (306). These properties are optimized for promoting the generation of high firing rates, up to hundreds of Hz (308). Mutations in KCNC1 have been identified in early onset DEE and in patients with intellectual disability without seizures (309, 310). Mutants have been functionally characterized in the Xenopus laevis oocytes. All characterized mutations resulted in partial or complete LoF, with some of them inducing negative dominance leading to >50%
reduction of current in heterozygosis (309–311). These results are consistent with hypo-
excitability of fast spiking neurons as the main pathogenic mechanism of KCNC1 DEE. Knock-out
Kcn1-/- mice have a subtle phenotype (312) and there are no mouse models engineered to carry
human KCNC1 mutations.

KCND1-3 encode the Kᵥ4.1-3 α subunits of the Shal family of the A-type voltage-gated K⁺
channels, which generate a rapidly inactivating outward K⁺ current and are important in
membrane repolarization in excitable cells (313). The \textit{de novo} heterozygous missense variant
p.V404M of KCND2 has been associated with DEE with onset at 2 months (314). Functional
analysis in \textit{Xenopus laevis} oocytes showed modified current kinetics and reduced inactivation,
consistent with GoF, but effects on neuronal excitability were not investigated (314).

2.4. Non-inactivating K⁺ channels

The ether-a-go-go (EAG) K⁺ channel family is formed by KCNH1 and KCNH5, which encode the
Kᵥ10.1 (EAG1) and Kᵥ10.2 (EAG2) channels, respectively. They generate non-inactivating voltage-
dependent K⁺ currents and in expression systems can form heteromeric channel complexes, in
which the slow activation of Kᵥ10.1 is dominant (315). KCNH1 heterozygous missense mutations
have been identified in patients with Zimmermann-Laband and Temple-Baraitser syndromes, as
well as in patients with unclassified syndromes and a broad phenotypic spectrum with intellectual
disability and epilepsy (316–320). Epilepsy is a key phenotypic feature in most patients with
KCNH1-related syndromes, who show both generalized and focal tonic-clonic seizures (319, 320).
Functional studies of KCNH1 mutants in cell lines have shown left-shifted voltage-dependence of
activation and slower deactivation kinetics, consistent with GoF (316, 317), although it is not clear
how this effect could cause the observed phenotypes. \textit{De novo} heterozygous missense variants of
KCNH5 have been identified in few DEE patients (321, 322). Functional analysis in transfected cell
lines has shown a strong hyperpolarizing shift of voltage-dependence of activation and an
acceleration of activation, consistent with GoF.

2.5. Ca\textsuperscript{2+} activated K\textsuperscript{+} channels

\textit{KCNMA1} encodes the pore-forming \(\alpha\)-subunit of the large-conductance \(\text{K}_{\text{Ca}1.1}\) \(\text{Ca}^{2+}\) activated K\textsuperscript{+} channel ("Big K\textsuperscript{+}", BK), which is activated by depolarizations and intracellular \(\text{Ca}^{2+}\) (323). \(\text{K}_{\text{Ca}1.1}\) is widely distributed in excitable and non-excitatory cells. Expression levels are highest in brain and muscle, where BK channels are critical regulators of neuronal excitability and muscle contractility.

In both excitatory and inhibitory neurons, BK channels are implicated in AP repolarization and after-hyperpolarization, influencing the shape, frequency, and propagation of APs (323). Heterozygous \textit{de novo} missense mutations in \textit{KCNMA1} are associated with a wide phenotypic spectrum primarily defined by brain and muscle dysfunction (324–326). \textit{KCNMA1}-linked DEE is characterized by a heterogeneous combination of epilepsy, dyskinesia, and intellectual disability.

Functional analysis in cell lines transfected with \textit{KCNMA1} mutations have shown both LoF and GoF effects that correlate with phenotypic features (325). While seizures do not show differential distribution between patients carrying GoF and LoF variants, neurodevelopmental and structural brain abnormalities are prevalent in patients with LoF mutations. There are no animal models carrying mutations identified in patients. \textit{Kcnma1}\textsuperscript{-/-} mice show ataxia, tremor, impaired coordination and spatial learning (327, 328). Pharmacological inhibition of \(\text{K}_{\text{Ca}1.1}\) channels induce tremor and ataxia in animals (329), but they might be used for treatment of GoF mutations.

2.6. Na\textsuperscript{+}-activated K\textsuperscript{+} channels

\textit{KCNT1} encodes the \(\text{K}_{\text{Na}1.1}\) subunit, which has a classical six transmembrane segments structure and forms a tetrameric Na\textsuperscript{+}-activated K\textsuperscript{+} channel (also called Slack, \(\text{KCa4.1}\) or Slo2.2). \(\text{K}_{\text{Na}1.1}\) is activated by both intracellular Na\textsuperscript{+} and voltage and is expressed in different organs, including the nervous system, heart, and kidney. In the central nervous system, it has a distinct expression
pattern with respect to \( K_{\text{Na}1.2} \) (encoded by the gene \( KCNT2 \)), but the two subunits can co-localize and form heteromeric channels (330, 331). \( K_{\text{Na}} \) channels can modulate intrinsic excitability and firing properties of different types of neurons. In particular, they contribute to generate the slow after-hyperpolarization that follows AP discharges, which induce \( \text{Na}^+ \) influx through \( \text{Na}_V \) channels (332).

Mutations in \( KCNT1 \) cause different phenotypes, including DEEs. \textit{De novo} heterozygous mutations were first identified in epilepsy of infancy with migrating focal seizures (EIMFS) also called malignant migrating partial seizures of infancy (MMPSI) (333). EIMFS is characterized by refractory focal seizures arising and status epilepticus with onset in the first six months of life. The neurological status progressively deteriorates, with progressive hypotonia, and severe development arrest. \textit{De novo} heterozygous mutations have been also identified in severe autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), sleep-related hypermotor epilepsy SHE (334) and other less common DEE phenotypes (335).

The identified mutations induce large GoF, increasing the \( K_{\text{Na}1.1} \) current amplitude (333, 336), while one heterozygous LoF variant (p.F932I) from a patient with severe generalized seizures and delayed myelination causes impaired \( K_{\text{Na}1.1} \) trafficking (337). Knock-in mice of the p.Y796H GoF \( KCNT1 \) mutation show early-onset seizures (338). Although \( K_{\text{Na}1.1} \) increases \( K_{\text{Na}} \) currents in both excitatory and inhibitory cortical neurons in primary cultures, an increase of the current in the subthreshold voltages is only found in inhibitory neurons, resulting in inhibitory neuron-specific impairments in excitability and AP generation. GoF \( KCNT2 \) mutations also cause DEE (339).

Blocking \( KCNT \) GoF mutants with quinidine, a class I antiarrhythmic drug, has shown variable success in patients because of dose-limiting off-target effects, poor blood-brain barrier (BBB) penetration, and low potency (335).

3. \( \text{Ca}^{2+} \) CHANNELS
Voltage-gated Ca\textsuperscript{2+} channels are important for numerous physiological functions; in neurons, they contribute to neuronal excitability and, at the synaptic level, mediate synchronous transmitter release. The voltage-dependent Ca\textsuperscript{2+} currents are generated by monomeric α1 subunits that have the canonical four domains of six transmembrane segments, (340, 341). The 10 cloned α1-subunits can be grouped into three families according to the membrane potential range at which the channel is activated and the sensitivity to drugs: the high-voltage activated dihydropyridine-sensitive channels (L-type; \textit{CACNA1S}/Ca\textsubscript{v}1.1, \textit{CACNA1C}/Ca\textsubscript{v}1.2, \textit{CACNA1}/Ca\textsubscript{v}1.3, and \textit{CACNA1F}/Ca\textsubscript{v}1.4), the high/moderate-voltage activated dihydropyridine-insensitive channels (P/Q-, N- and R- type; \textit{CACNA1A}/Ca\textsubscript{v}2.1, \textit{CACNA1B}/Ca\textsubscript{v}2.2 and \textit{CACNA1E}/Ca\textsubscript{v}2.3), and the low-voltage-activated (T-type; \textit{CACNA1G}/Ca\textsubscript{v}3.1, \textit{CACNA1H}/Ca\textsubscript{v}3.2 and \textit{CACNA1I}/Ca\textsubscript{v}3.3) channels. It has been proposed that L-type channels may be implicated in the generation of epileptiform paroxysmal depolarization shifts (342) and that they may be therapeutic targets for epilepsy (343). However, besides Timothy syndrome, a severe multiorgan disorder caused by \textit{CACNA1A}/Ca\textsubscript{v}2.1 GoF mutations (in which surviving patients can develop epilepsy and autism) (344), there are no reports of their direct involvement in DEE. At least for high-voltage activated channels, functional properties, and intracellular trafficking of α1 subunits can be modulated by accessory subunits, including the β-, α2δ-, and γ-subunits (345).

\textbf{3.1 T-type channels}

T-type channels (\textit{CACNA1G/H/I}, Ca\textsubscript{v}3.1-3) are widely expressed throughout the nervous system (346). Voltage-dependent opening of T-type channels occurs at comparatively negative membrane potentials and, depolarizing the cell membrane, facilitates the generation of APs. This function is especially relevant in several central neurons in which T-type channels are particularly abundant in dendrites, where they enhance subthreshold postsynaptic potentials and facilitate the propagation of the electrotonic signal to the cell body (347). They are also involved in the firing of
rebound burst of APs that support various forms of neuronal pacemaking, particularly in the thalamocortical network, whose dysfunction is implicated in absence seizures (348). Polymorphisms in CACNA1G/CaV3.1 and CACNA1H/CaV3.2 causing in general mild GoF have been identified as risk factors for idiopathic generalized epilepsy (349). De novo heterozygous missense variants in CACNA1G/CaV3.1 and CACNA1H/CaV3.1 have been recently identified in patients with infantile-onset DEE (350, 351) and variable neurodevelopmental phenotypes including DEE with cognitive impairment, hypotonia, and epilepsy (352). Functional effects are consistent with GoF (including persistent Ca²⁺ current at resting membrane potential for CACNA1H/CaV3.1 variants), or mixed GoF and LoF. Evidence for the involvement of CACNA1H/CaV3.2 in DEE is more limited. Gene targeted animal models of T-type channels DEE mutations are not available yet.

3.2. P/Q-type Ca²⁺ channels

CACNA1A encodes the Ca₂⁺₁.₁ Ca²⁺ channel, which generates the high voltage-activated P/Q-type Ca²⁺ current with moderate voltage-dependent inactivation. CACNA1A is widely expressed in the central nervous system and is essential for fast and synchronous neurotransmitter release in numerous neuronal subtypes (353). CACNA1A mutations have been associated with episodic ataxia type 2 (EA2), spinocerebellar ataxia type 6 (SCA6), familial hemiplegic migraine type 1 (FHM1) (354–356) and, more recently, with severe intellectual disability, motor impairment, and episodic ataxia (61, 357). Functional characterization of CACNA1A DEE mutations performed in transfected cell lines has generated controversial results because both LoF (decreased channel targeting at the cell membrane) and GoF (hyperpolarized shift of the activation curve) effects were reported (358). A mouse model of CACNA1A DEE has not been generated yet.

3.3. N-type Ca²⁺ channels

CACNA1B encodes the Ca₂⁺₂.₂ N-type Ca²⁺ channel, which generates the high voltage-activated Ca²⁺ current with moderate voltage-dependent inactivation. CACNA1B is expressed throughout the
central nervous system and acts synergistically or complementarily with CACNA1A for generating
presynaptic Ca\(^{2+}\) fluxes that mediate neurotransmitter release (353). Expression of CACNA1B is
thought to be crucial for neurotransmission in the early postnatal period as Ca\(_{v}2.2\) channels are
replaced by CaV2.1 channels in most mature synapses (353). In particular, Ca\(_{v}2.2\) is implicated in
asynchronous synaptic release, which occurs up to tens of seconds after the AP (353). Ca\(_{v}2.2\) may
also have a role in synaptic plasticity, synaptogenesis, gene transcription, neuronal survival, and
migration of immature neurons (340). CACNA1B variants have been identified in a severe form of
autosomal recessive DEE featuring developmental delay, microcephaly, inability to walk or speak,
early onset refractory seizures, myoclonus/dyskinesia, frequent feeding difficulties and high risk of
premature demise (359). The identified variants are predicted to truncate the Ca\(_{v}2.2\) protein,
leading to LoF and haploinsufficiency, although the genotype/phenotype relationships are not
clear. There are no gene targeted mouse models of the identified human variants, but Cacna1b\(^{-/-}\)
mouse models show overt neurodevelopmental abnormalities, including abnormal locomotor
activity and memory impairment (360).

3.4. R-type Ca\(^{2+}\) channels

CACNA1E encodes the moderate voltage-activated Ca\(_{v}2.3\) Ca\(^{2+}\) channel, which generates the R-
type Ca\(^{2+}\) current with fast voltage-dependent inactivation. It is highly expressed in the central
nervous system and involved in generating the Ca\(^{2+}\) influx in synaptic terminals that initiates rapid
release of neurotransmitters. Mutations in CACNA1E cause severe autosomal dominant DEE with
macrocephaly, hypotonia, early-onset refractory seizures, profoundly impaired neurodevelopment
and hyperkinesia (361). Functional analysis of CACNA1E variants in transfected cell lines mostly
showed GoF effects with facilitated activation and slowed inactivation (361), consistent with
increased neurotransmitter release and network hyperexcitability. Other variants were predicted
to generate truncated non-functional proteins and haploinsufficiency. *Cacna1e*−/− null knock-out mice do not show overt neurological phenotypes (362).

### 4. OTHER CATION CHANNELS

#### 4.1 Voltage-independent Na⁺ channels

The sodium leak NALCN channel (NaVI2.1) is predominantly expressed in neurons where it is important for setting resting potential and controlling neuronal excitability. *NALCN* encodes for a voltage-independent, non-inactivating cation channel permeable to Na⁺, K⁺, and Ca²⁺ that generates the background TTX resistant Na⁺ leak current (363, 364). Autosomal-recessive missense and nonsense *NALCN* mutations have been identified in a DEE with onset at birth or in early infancy, characterized by variable degrees of hypotonia, speech impairment and intellectual disability (infantile hypotonia with psychomotor retardation and characteristic facies, IHPRF) (365). A subsequent study found heterozygous de novo *NALCN* missense variants to cause congenital contractures of the limbs and face, hypotonia, and developmental delay (CLIFAHDD) (366, 367). Functional studies in transfected cell lines have generated controversial results, showing GoF, LoF or dominant-negative effects for both syndromes (367, 368).

*Nalcn*−/− knock-out mice show severely disrupted respiratory rhythm and die within 24 hours of birth.

#### 4.2 Hyperpolarization-activated cyclic nucleotide-gated channels (HCN)

HCN are Na⁺/K⁺ permeable channels that are activated by hyperpolarization at voltages more negative than -50 mV (369). cAMP and cGMP directly bind to the intracellular cyclic nucleotide-binding domain and shift the activation curve of HCN channels to more positive voltages, increasing channel activity. The four known HCN isoforms (HCN1-4) have secondary structure that resembles that of K⁺ channels. HCN1 and HCN2 are the main isoforms expressed in the brain with a predominant somato-dendritic expression. The hyperpolarization-activated currents generated by HCN channels in neurons, named *Ih* or *Iq*, are depolarizing and contribute to set the resting
membrane potential, shape synaptic inputs and generate rhythmic and synchronized neuronal activity implicated in pacemaking and somato-dendritic oscillations (370).

*De novo* dominant missense *HCN1* variants were initially identified in DEE patients with a Dravet-like phenotype (371), but have subsequently been related to a wider phenotypic spectrum ranging from mild generalized epilepsy or GEFS+ to severe early onset DEE (372). Functional studies in cell lines identified GoF as the major pathophysiological mechanism and a LoF with dominant negative effect for a limited number of variants (371). Knock-out *HCN*+/− mice show deficits in motor learning and modifications of spatial memory, but not a DEE phenotype (373) while the knock-in mouse for the p.M305L mutation shows DEE (374). Functional analysis in CHO cells and pyramidal neurons demonstrated that the p.M305L mutation causes a constitutive activation of the channel (372).

### 5. The Na⁺/K⁺-ATPase pump

The Na⁺/K⁺-ATPase (NKA) ion pump is a ubiquitous transmembrane enzyme responsible for active exchange of Na⁺ and K⁺ ions across the plasma membranes in higher eukaryotic cells and is composed of a large catalytic α-subunit and smaller β- and γ-subunits that modulate the membrane exposure and activity of α-subunits (375, 376). In neural tissue, NKA generates an outward current that contributes to the resting membrane potential, and powers secondary active transports, including Na⁺/H⁺ and Na⁺/Ca²⁺ exchanges, K⁺/Cl⁻ cotransport, and Na⁺-dependent neurotransmitter uptake (375).

The four known human isoforms of the α-subunit (α1-4), encoded by four paralogous genes (*ATP1A1-4*) have developmental and tissue expression specificity. The α2- and α3-isoforms, encoded by *ATP1A2* and *ATP1A3*, are predominantly expressed in the central nervous system. Constitutional heterozygous mutations in *ATP1A2/A3* have been associated with several autosomal dominant neurological disorders including familial hemiplegic migraine (FHM), rapid-
onset dystonia-parkinsonism (RDP), alternating hemiplegia of childhood (AHC), cerebellar ataxia-
areflexia-progressive optic atrophy (CAPOS), and relapsing encephalopathy with cerebellar ataxia
(RECA) (377–379). Epilepsy and intellectual disability may co-occur with FHM and AHC, and severe
epilepsies have been described in rare patients with ATP1A2 or ATP1A3 mutations (380, 381).
Early lethal hydrops fetalis, intrauterine growth restriction, arthrogryposis, microcephaly,
polymicrogyria, and lack of respiratory drive have been associated with homozygous truncating
mutations in ATP1A2 (382, 383). Investigating the genetic causes of DEEs variably associated with
malformations of cortical development in a large cohort of patients, Vetro and collaborators
identified 22 patients carrying de novo or inherited heterozygous ATP1A2/A3 mutations. Most
patients manifested early, often neonatal, onset seizures with a multifocal or migrating pattern. A
distinctive, ‘profound’ phenotype, featuring polymicrogyria or progressive brain atrophy and
epilepsy, resulted in early lethality. Neuropathological analysis of the whole brain in two
individuals with polymicrogyria revealed a mainly neural pathogenesis, compounded by vascular
and leptomeningeal abnormalities (384). Functional studies performed in COS-1 cells transfected
with different mutations demonstrated a LoF mechanism, with a wide continuum of severity
distributed across mutations that variably impaired NKA pump activity. Mutations associated with
most severe phenotypes cause lack of COS-1 cell-survival (384). Interestingly, PRRT2, a gene
whose deficiency is associated with paroxysmal disorders including epilepsy (see next section) is
co-expressed with and is a physiological activator of α3-NKA in several brain areas (385).

VIII. DYSFUNCTIONS IN SYNAPTIC TRANSMISSION AND PLASTICITY

Although the most straightforward causes of hyperexcitability have been historically and widely
associated with mutations in ion channel genes, dysfunctions of synaptic transmission are
emerging as primary causes of epilepsy and DEE. That is why the term “synaptopathies” was
coined, inherently meaning that anomalies of information transfer at the synapse can profoundly
affect network excitability, excitatory/inhibitory (E/I) balance and nervous system development,
triggering epileptogenesis.

In the last 30 years, the machinery of synaptic transmission has been largely clarified in the most
subtle molecular details. Several physiologically important molecular actors orchestrate
neurotransmitter (NT) release at the presynaptic side and decode its message into a biological
response at the postsynaptic side. Biology teaches that the more important a process is, the higher
the complexity is involved in its control. Thus, there is a relatively large probability that gene
mutations hits some of these numerous actors.

Neurotransmitter release evoked by APs reaching nerve terminals is based on the exocytotic
fusion of small organelles, synaptic vesicles (SVs), real nanomachines that sense Ca^{2+} entry through
presynaptic voltage-dependent channels and trigger exocytosis by activating a SNARE-mediated
fusion machine that incorporates the SV in the presynaptic membrane and releases its content
into the synaptic cleft. The SV-competent membrane patch is eventually retrieved by distinct
endocytotic mechanisms that take place at distinct activity patterns. Endocytosis regenerates new
SVs that are loaded by NT by an active proton gradient generated by the SV-associated vacuolar
H^{+}-adenosine triphosphatase (vATPase) (386, 387). Synaptic vesicles are organized into distinct
functional pools, including (i) the Readily Releasable Pool (RRP), comprising SVs immediately
recruited for exocytosis, (ii) a Recycling Pool (RP) made by SVs that can be rapidly recruited to
replenish the RRP depleted upon activity, and (iii) a Resting Pool (ResP) representing a reserve of
SVs that are not immediately releasable upon activity (388, 389).

When referring to alterations in synaptic transmission and plasticity, some issues should be
considered. Except for postsynaptic receptors and scaffolding proteins, the presynaptic
components of excitatory and inhibitory synapses share most of the actuators of NT release and,
in principle, should be equally affected by mutation-induced protein dysfunction, without net
changes in the excitatory/inhibitory balance. However, differentially expressed proteins or the
different impact of protein dysfunction in excitatory and inhibitory neurons very often imply an
overt phenotype affecting network excitability and/or the excitatory/inhibitory balance. For
example, a protein involved in SV recycling and refilling of RRP will be more essential for the
proper functionality of high-frequency firing neurons (e.g., the PV-positive interneurons) than for
excitatory neurons.

Another important issue is the respective weight of basal synaptic strength and short-term
plasticity mechanisms. Short-term plasticity is a fundamental determinant of network activity and
excitability and thereby plays a central role in epileptogenesis. As neurons do not fire single action
potentials (APs), but rather trains of APs, short-term plasticity phenomena, such as depression or
facilitation, have a strong impact on the network computational activities, including frequency
band filtering of synaptic inputs or pattern detection activities (390, 391).

Synaptic transmission is a major determinant of excitability at the neuronal network level. Intrinsic
excitability at single neuron level is mostly affected by the size of the neuron and its input
resistance, and by the level of expression and functionality of voltage-dependent ion channels,
particularly at the axon initial segment, the AP initiation spot. However, at the level of a
population of synaptically connected neurons, intrinsic excitability is not the only factor defining
the firing and bursting behavior of the network, as well as its synchrony. While inhibitory synapses
act to confine the excitation waves temporally and spatially, excitatory synaptic connections play a
prominent role in establishing the activity of the network, owing to their short-term plasticity
properties (392).

Mutations in numerous genes affect synaptic functions, and the number of so-called
“synaptopathies” is continuously raising. Strong evidence, accumulated in the last two decades,
has shown that mutations in fundamental actors of the complex process of NT release can result in
neurodevelopmental disorders with epilepsy (FIGURE 8). In this section, we will only consider mutations that affect purely synaptic genes, leaving mutations in the genes of presynaptic and postsynaptic ion channels to the dedicated chapter. Among genes encoding for bona fide synaptic proteins, a distinction should be made between expression/function alterations affecting fundamental proteins for the synaptic transmission machinery, and genes that play a modulatory action on the process by impacting more on synaptic plasticity than on the essential synaptic machinery. In addition, the targets of mutations can participate in distinct processes contributing to synaptic transmission. According to the view of the tetrapartite synapse, four distinct entities are involved in the physiological regulation of synaptic transmission: the presynaptic neuron, the target postsynaptic neuron, the extracellular matrix and the perisynaptic astrocyte. While the impact of mutations in astrocyte-specific genes is still poorly understood, we can consider three main classes of synaptopathies, namely: (i) presynaptic synaptopathies, including defects of the post-docking SV priming/fusion processes, as well as defects in the processes regulating SV trafficking or NT synthesis and loading into SVs; (ii) postsynaptic synaptopathies, including defects in postsynaptic receptors and their scaffold/transduction systems; and finally (iii) extracellular synaptopathies, including defects in trans-synaptic and extracellular matrix (ECM) proteins at the synaptic cleft and secreted synaptic proteins (FIGURE 8). For all these classes of synaptic pathologies, the identification of gene mutations in patients has catalyzed an extensive research activity that often contributed to clarifying the pathomechanism of the disease and elucidating the physiological role of the synapse-specific gene products. A substantial contribution to physiopathology of synapse-bases DEEs came from the exploitation of murine knockout or knock-in experimental models, recapitulating at least some traits of the clinical phenotypes, as well as from mutation studies in transfected cell lines and primary neurons, up to the studies performed in iPSC-derived neurons either containing the patient’s mutation in the patient’s own genetic
background or in which the mutation under study has been reproduced by CRISPR-Cas9 gene editing in WT cells. There is a wide spectrum of phenotypic DEE features stemming from alterations of synaptic function, although genotype-phenotype correlations are not always straightforward (393). We will follow the aforementioned classification to list the main DEE-related genes.

1. Presynaptic synaptopathies

Mutations in many genes encoding proteins involved in the multi-step process of NT release cause epilepsy. Starting from the machinery essential for regulated exocytosis, mutations in genes encoding t-SNARE proteins Syntaxin 1B, SNAP25b and VAMP/synaptobrevin, the SNARE-associated proteins STXBP1 (Munc18-1) and PRRT2, the a-subunit of the P/Q type voltage-dependent Ca\(^{2+}\) channel, and the critical SV Ca\(^{2+}\) sensor synaptotagmin have been identified and associated with epilepsy and DEEs (9, 358, 359, 394–400). In mice, constitutive knockout of these genes greatly hinders AP-driven NT release, with a compensatory increase of spontaneous release and synaptic facilitation (401–407). However, it remains unclear how an impairment of ubiquitous players of evoked NT release can trigger epileptogenesis, presumably by their different impact on distinct neuronal populations, resulting in E/I imbalance and circuit instability. Similar mechanisms are likely to take place in case of mutations in genes encoding proteins not directly actuating NT release, but supporting trafficking, maintenance, and integrity of the SV pools. Loss of function in these proteins, which include the SV proteins synapsins and SV2 and the proteins actuating the endocytotic retrieval of SVs after each round of exocytosis, such as dynamin or amphiphysin, may induce network hyperexcitability by affecting, to a larger extent, neurons undergoing high frequency activity, such as parvalbumin (PV)-positive inhibitory neurons.

1.1. Perturbation of priming/fusion event and of Ca\(^{2+}\) sensitivity
SNARE PROTEINS. Mutations in the large family of genes encoding membrane proteins mediating vesicle fusion, the so-called SNARE proteins SNAP-25, Syntaxin-1 and VAMP2, constituting the fusion machine actuating exocytosis have been reported. Mutations in the two SNARE motifs of SNAP-25, particularly in its splicing isoform SNAP-25b cause a combination of epilepsy and cognitive deficits that are reproduced by the selective KO of SNAP-25b in mice (395, 408–410). The second presynaptic SNARE protein, syntaxin-1, has been less implicated in epilepsy and DEE. However, some mutations associated with febrile epilepsy have been mapped to the Habc region of the Syntaxin-1b isoform (STX1B) that controls its open/close conformation permitting the assembly of the SNARE complex. In mice, the a and b isoforms of Syntaxin-1 are redundant, so that the single KO mice are viable. However, the genetic “freezing” of the b isoform in the open conformation in the syntaxin-1a KO generates severe seizure activity and premature death (402, 411). Mutations in the SNARE motif of VAMP2 were also identified in patients with intellectual disability, epilepsy and hyperkinetic movement disorder (396). While it is conceivable that inactivation of SNARE proteins, profoundly altering neuronal signaling at the synapse, can cause neurodevelopmental deficits, the mechanism of the frequently associated hyperexcitability and epilepsy is more elusive. Notably, the three SNARE proteins are the specific targets of Tetanus and Botulinum neurotoxins (412) and either toxin-mediated or genetic inactivation of either SNARE protein irreversibly blocks evoked neurotransmitter release. The different sensitivities of excitatory and inhibitory neurons to SNARE inactivation is believed to generate an E/I imbalance resulting in the developmental deficits.

CALCIUM SENSING/TRIGGERING MACHINERY. Synaptotagmin1/2 is the fundamental Ca$^{2+}$-binding sensor for evoked fast and synchronous NT release. Synaptotagmin binds to SNARE proteins together with complexins limiting spontaneous release and, bearing two C2 domains, acts as a low affinity Ca$^{2+}$ sensor that is exposed to high concentrations of Ca$^{2+}$ entering through activated...
voltage-gated Ca\textsuperscript{2+} channels and upon Ca\textsuperscript{2+} binding trigger membrane deformation and fusion (413, 414). Five heterozygous mutations were reported in Synaptotagmin-1 located at the Ca\textsuperscript{2+} and phospholipid binding motifs of the second C2 domain (C2B) in patients presenting with various degrees of developmental delay and EEG abnormalities, but no epilepsy (415). Synaptic transmission was impaired in neurons expressing the synaptotagmin mutants, with graded dominant-negative effects that could be rescued by K\textsuperscript{+} channel antagonists (400). Presynaptic voltage-gated Ca\textsuperscript{2+} channels (VGCC) Cav2.1 and Cav2.2, corresponding to the P/Q- and N-type VGCCs are the essential electro-chemical transducers concentrated in the nano- and micro-domains of the active zones and converting APs into synchronous exocytosis of SVs. PRRT2 is a neuron-specific, type-2 membrane protein with a C-terminal anchor that concentrates in synaptic and axonal domains, where it interacts with key components of the fusion/Ca\textsuperscript{2+} sensing machinery (synaptotagmin 1/2, SNAP-25 and VAMP2) boosting the Ca\textsuperscript{2+} sensitivity of NT release and suggesting a function in the Ca\textsuperscript{2+}-dependent transition from SV priming to fusion (398). As a consequence, the probability of release at excitatory synapses is dramatically decreased, with a parallel marked increased synaptic facilitation that results in an E/I imbalance and lack of network stability (406, 416, 417). This role of PRRT2 has recently been supported by the discovery of a direct interaction of PRRT2 and P/Q VGCCs that contributes to concentrate them at the nanodomain where the machinery for synchronous release is assembled. In the absence of PRRT2, membrane targeting and concentration of P/Q channels at active zones is impaired, decreasing Ca\textsuperscript{2+} influx in response to APs (418). PRRT2 has been identified as the causative gene for several paroxysmal neurological disorders including epilepsy, paroxysmal kinesigenic dyskinesia, but also migraine and ataxia. These disturbances have been associated with a severe encephalopathic phenotype with intellectual disability in the few patients with homozygous or compound heterozygous mutations (398).
Another SV protein playing an important role in regulating the initial release probability of SVs and neural network synchronous activity is the Synaptic Vesicle glycoprotein-2 (SV2). SV2 is encoded by three paralog genes (SV2A, SV2B, SV2C) with distinct patterns of expression in neuronal populations. SV2 plays multiple roles as a catalyzer of evoked NT release: it accelerates SV priming, increases the size of the RRP, regulates the stability and trafficking of synaptotagmin, boosting the Ca^{2+} sensitivity of release. Interestingly, SV2A is essentially expressed in inhibitory neurons and its loss-of-function impairs synaptic inhibition. Accordingly, individuals bearing point mutations in the SV2A gene experience epilepsy and cognitive deficits (419, 420).

SV PRIMING MACHINERY. The preparation of SVs for fusion after docking is an essential mechanism for allowing fast and synchronous release. Two proteins are essential for this progression, Munc-18 (STXBP1) and Munc-13 (UNC13A), while other nerve terminal proteins such as RIM1, SV2 and synapsin I have a modulatory role. The Munc-18 and Munc-13 tandem is needed for a correct assembly of the SNARE complex, whereby Munc-18 regulates the participation of syntaxin-1 to the complex, while Munc-13 activates syntaxin-1, stabilizes the active zone-SNARE-SV assembly and protects the SNARE complex from disassembly by the ATPase NSF (421, 422).

While a nonsense mutation in UNC13A was identified in a single patient with microcephaly and interictal multifocal epileptiform EEG activity (423), mutations in STXBP1 were repeatedly identified in a series of epileptic encephalopathies including Ohtahara, West, and Rett syndromes. Over 85 distinct STXBP1 mutations are known and de novo STXBP1 mutations are among the most frequent causes of DEEs of synaptic origin with severe intellectual disability (424).

ORGANELLE ACIDIFICATION AND NT LOADING. As mentioned above, vATPase plays a fundamental role in the active loading of SVs with the surprisingly reproducible amount of NT that constitute the “quantum”. Quantal reproducibility relies on the fact that the amount of NT is an important determinant of the probability of release, so that only fully filled SVs are likely to be released (425,
This mechanism is based on the dissociation of the V1 catalytic cytosolic domain from the V0 transmembrane proton transfer domain upon complete build-up of the proton gradient and corresponding NT loading. The synaptic protein encoded by the DMXL2 gene, rabconnectin-3a, is strictly involved in the assembly and incorporation of vATPase into SVs (427, 428). The role of v-ATPase in pH homeostasis and intracellular signaling pathways is ubiquitous, with a prominent role in the nervous system. In humans, mutations in 16 of the 22 genes encoding for vATPase subunits are associated with a variety of congenital disorders with neurological impairment. Recently, four different de novo missense mutations in ATPV1A have been associated with a clinical spectrum of DEE ranging from rapidly progressive early encephalopathies to mild intellectual disability with epilepsy (429). Unexpectedly, rather than NT loading, the identified pathomechanism involves dysfunctions of lysosomal homeostasis impacting on neuronal connectivity. Missense pathogenic variants of ATPV0A1, which participates in the transmembrane proton pumping machinery of vATPase, are also associated with DEE (430). Heterozygous copy number variations and loss-of-function biallelic mutations in DMLX2, regulating the trafficking and activity of v-ATPase, are associated with a severe DEE (Ohtahara syndrome) with a superimposable pathomechanism consisting of defective endolysosomal homeostasis and autophagy resulting in synaptic loss (431).

1.2. Perturbation of synaptic vesicle trafficking within nerve terminals

Synaptic vesicles undergo a restless cycle within nerve terminals aimed at preserving their availability in a sufficient amount in the RRP and maintaining synaptic transmission also during high frequency activity. Thus, loss-of-function of any of the vast cohort of nerve terminal proteins operating SV endocytosis and maintaining the recycling pool of SVs are predicted to impact on SV availability and impair NT release in the tonic high frequency discharging neurons (387). Since neurons with these characteristics are often inhibitory interneurons (such as the PV-positive neurons) a diffuse impairment in SV endocytosis/recycling often results in an E/I imbalance.
A large array of gene products mediating various steps of the endocytosis process has been associated with epilepsy and cognitive impairment. These include the membrane adaptor AP-2, synaptophysin (SYP), dynamin-1 (DNM1), the coat protein clathrin (CLTC), as well as proteins involved in activity-dependent bulk endocytosis such as Rab11, TBS1D24 and AP-1. In the case of AP-2, four patients bearing a point mutation in the µ2 subunit of the protein presented with epilepsy and developmental delay (432). Synaptophysin is an integral SV protein interacting with VAMP2 and regulating its availability and retrieval during endocytosis (433). Nonsense and missense mutations in the X-linked SYP gene have been associated with epilepsy and cognitive defects (434, 435). *De novo* mutations in the DNM1 gene result in DEEs including West and Lennox-Gastaut syndromes (436). Dynamin-1 is the essential GTPase that mediates SV fission by assembling in a collar around the neck of the budding vesicle and inducing constriction thanks to the mechanical force produced by GTP hydrolysis (437). Most of the DNM1 mutations identified thus far cluster within the GTPase domain (436). A series of missense and nonsense mutations in the CLTC gene encoding for clathrin, the building block of the endocytic coat that allows to increase the curvature of the vesicle bud to form the endocytic vesicle, have been identified in patients with epilepsy and intellectual disability (438, 439). Impairment in bulk endocytosis also occurs with mutations in AP-1, Rab11 and TBC1D24. This gene, whose mutants are associated with epilepsy and DOORS (Deafness, Onychodystrophy, Osteodystrophy, mental Retardation and Seizures) syndrome affect several processes linked to neural development and synaptic transmission, such as endocytosis, endosome/lysosome flux and neurite growth (440–444).

Synapsins (Syns) constitute a family of three synaptic genes (SYN1, SYN2 and SYN3) that encode for SV-associated proteins regulating SV formation, SV pool maintenance and SV trafficking between the functional SV pools. While Syn3 is a developmental isoform and is downregulated after birth, Syn1 and Syn2 represent the adult isoforms (445–448). Knockout mice for Syn1...
(homozygous females or hemizygous males), Syn2, Syn1/Syn2 and Syn1/Syn2/Syn3 all exhibit an epileptic and autistic-like phenotype with impairment of social interactions and cognitive functions (449, 450). As observed in other mouse models (e.g., Grin1 and Grin2b), heterozygous mice do not show an overt phenotype. An array of nonsense and missense mutations have been identified in patients with epilepsy and/or ASD. Most nonsense mutations in the X-linked gene SYN1 have been identified in epilepsy patients, whose seizures were typically triggered by contact with water regardless of the water temperature. Developmental delay of a variable degree was frequently associated (451–456). Owing to their role in regulating network activity and stability, presynaptic proteins can also represent a target for antiepileptic drugs, such as levetiracetam that binds to SV2 on SVs (457).

2. Postsynaptic and extracellular synaptopathies

Neurotransmitters released by the pre-synaptic terminals diffuse in the extracellular space and transfer information downstream by interacting with specific post-synaptic receptors. Mutations have been identified in genes encoding receptors for the main excitatory (glutamate) and inhibitory (GABA) neurotransmitters, as well as in transporters involved in ionic homeostasis and neurotransmitter reuptake, receptor-associated scaffolding proteins and extracellular trans-synaptic proteins.

2.1 Ionotropic neurotransmitter receptors

NMDA GLUTAMATE RECEPTORS. NMDA glutamate receptor subunits are encoded by the GRIN gene family, formed by seven genes: GRIN1 (encoding the GluN1 subunit), GRIN2A-D (encoding the GluN2A to D subunits), and GRIN3A-B (encoding the GluN3A and B subunits). Receptor activation requires binding of both glutamate and glycine, which are often considered co-agonists. Functional NMDA receptors are heterotetramers composed of two GRIN1 subunits binding glycine and two GRIN2 or GRIN3 subunits binding glutamate. They are essential for numerous
physiological functions, including neuronal migration, synaptic connectivity, neuronal pruning and survival, and synaptic plasticity. They are Ca\(^{2+}\)-permeable and generate a slow voltage-dependent synaptic current.

Among NMDA receptor subunits, \textit{GRIN1}, \textit{GRIN2A} and \textit{GRIN2B} are the target of most variants identified in patients with neurodevelopmental disorders. Epilepsy in patients carrying \textit{GRIN} mutations has onset from birth to few years of early childhood. Mutations can be either inherited or \textit{de novo} and generate a spectrum of phenotypes ranging from mild intellectual disability to severe DEE (458). The \textit{GRIN1} spectrum includes LoF of varying severity with a dominant negative effect and is characterized by severe intellectual disability with absent speech, seizures (in about 65% of patients), hypotonia, dyskinesia, cortical blindness and generalized cerebral atrophy (63). \textit{GRIN2A} mutations have either LoF and GoF effects and are associated with epilepsy and intellectual disability with normal brain imaging (58, 459, 460). Seizures often originate in the temporo-rolandic regions, and EEGs often show centrotemporal spike-wave discharges, which may be continuous during slow-wave sleep (CSWS or CSWSS). Affected individuals exhibit a range of language/speech problems and, at times, complete aphasia (458). The \textit{GRIN2B} spectrum, characterized by various GoF and LoF mechanisms, is similar to the \textit{GRIN1} spectrum and includes developmental delay, hypotonia, epilepsy (in about 50% of patients), movement disorders and, at times, cortical malformations (461). The phenotypic range of \textit{GRIN2D} missense GoF variants includes developmental delay with failure to thrive, intellectual disability, hypotonia, and hyperreflexia (59).

Heterozygous and homozygous knock-out (null) mouse models have been generated for each of the seven \textit{GRIN} genes (458). Mice carrying homozygous null mutations for \textit{Grin1} and \textit{Grin2b} are postnatal lethal, whereas heterozygous mice survive normally, but they have not been characterized in detail. Also, knock-in mice carrying mutations identified in patients have been
generated (462–464). Grin2α<sup>S/644G</sup> and Grin2α<sup>/N615K</sup> mice show perinatal lethality in homozygosis, increased seizure propensity, behavioral and cognitive deficits in heterozygosis. Grin2b<sup>+/C456Y</sup> mice show anxiety-like behavior with strongly reduced Grin2b levels and NMDA currents.

AMPA GLUTAMATE RECEPTORS. AMPA glutamate receptors are composed by four types of subunits: GluA1–GluA4 subunits (also named GluR1-4) encoded by the GRIA1–GRIA4 genes. Most AMPARs are heterotetrameric, consisting of symmetric 'dimer of dimers' of GRIA2 and either GRIA1, GRIA3 or GRIA4 (465, 466). AMPA receptors interact with multiple accessory proteins (e.g., TARP and cornichon). Glutamate released from the presynaptic terminal triggers the rapid opening of AMPA receptor channels that generate an inward cation current. The GRIA2 mRNA is often edited at the p.Q607 residue that confers Ca<sup>2+</sup> impermeability to mature receptors containing the edited GluA2 subunit. The AMPA current underlies most of the excitatory synaptic signaling in the central nervous system. It is typically brief (on the order of a few ms) because glutamate rapidly unbinds and is removed from the synaptic cleft and leads to a brief depolarization of the postsynaptic neuron. De novo heterozygous GRIA2 mutations have been found in patients with different associations of seizures, speech impairment, intellectual disability and autistic features (467) with both GoF and LoF mechanisms. The most severe phenotypes were associated with the p.A639S mutation, which caused DEE with death in infancy. There are no mouse models available for human mutations. Gria2<sup>−/−</sup> mice exhibit increased mortality, impaired motor coordination and behavioral abnormalities, whereas heterozygous mice do not show an overt phenotype (468). De novo heterozygous missense mutations in GRIA3, as well as genomic deletions, have been initially identified in a form of X-linked intellectual disability with dysmorphic features, a relatively mild phenotype due to a LoF mechanism (469). However, GRIA3 mutations have been associated with a larger phenotypic spectrum that also includes severe early onset DEE (470). There are no animal models that carry human mutations. Finally, de novo heterozygous variants in GRIA4 have been
identified in a range of phenotypes characterized by variable developmental delay, ranging from mild to severe, with absent speech, epilepsy, gait abnormalities, and behavioral problems (471).

GABA RECEPTORS. Ionotropic GABA (GABA_A) receptors are pentameric assemblies of up to 3 of 19 subunits encoded by distinct GABR genes (GABRA1–6, GABRB1–3, GABRG1–3, GABRD, GABRE, GABRP, and GABRQ). The combination of two α-subunits (GABRA), two β-subunits (GABRB) and one γ-subunit (GABRG) is the most common functional receptor in the brain. GABA_A receptors are cys-loop ligand-gated chloride/anion channels that, at low intracellular chloride, implement an outward inhibitory current generating hyperpolarization and decrease in membrane impedance by a shunting effect. The GABAergic synaptic action is essential for reducing excitability in neuronal networks and generating rhythms of activity. The synaptic GABA_A receptors provide brief but strong phasic inhibition, whereas those extrasynaptic receptors can induce long-lasting tonic effects in response to ambient GABA. GABRA1, GABRA2, GABRA3, GABRA5, GABRB1, GABRB2, GABRB3 and GABRG2 genes have been identified as targets of de novo heterozygous DEE mutations (472). Mutations in GABRA2, GABRA3, GABRA5, and GABRB1 have been mostly associated with severe phenotypes, whereas variants in other GABA_A genes include, in addition to DEE, phenotypes associating moderate/mild intellectual disability with epilepsy, and familial epilepsy without intellectual disability (16, 473–475). The severe phenotypes of GABRA1 DEE mutations share some of the clinical features of Dravet syndrome (476). Most GABA_A mutations induce LoF (with dominant negative effects), by decreasing membrane targeting or modifying gating properties or GABA sensitivity (474, 475, 477–479). Overall, LoF of GABA_A receptors reduce the inhibitory tone in neuronal networks and thus generate hyperexcitability; the effect has some similarity to that of SCN1A mutations that reduce the intrinsic excitability of GABAergic neurons. A different functional effect has recently been proposed for mutations in GABRD, which encodes the GABA_A δ-subunit found in extra-synaptic receptors generating tonic GABA_A currents (480). DEE
patients carrying mutations in this gene have generalized epilepsy, intellectual disability, and
behavioral problems.

Functional analysis of the identified mutations showed GoF of GABA_{A} receptors containing the δ-
subunit with increased GABA_{A} current. Thus, increased tonic GABA_{A}-evoked current may be a
novel pathological mechanism in DEE and neurodevelopmental diseases. Electroclinical findings in
these patients resembled those reported in patients carrying LoF mutations in the GABA uptake
transporter SLC6A1/GAT1 (481), consistent with a similar pathogenic mechanism (see below).

Heterozygous Gabra1 knockout mice show spontaneous electrographic spike-wave discharges
with behavioral absence-like seizures and develop myoclonic seizures later in life, consistent with
a relatively mild IGE phenotype (482). Gabrb2 knockout mice do not show spontaneous epilepsy,
but are more susceptible to seizures and exhibit behavioral disturbances (483). Heterozygous
Gabrb3 knockout mice show epileptic seizures, EEG abnormalities and a range of behavioral
deficits (484, 485). Homozygous Gabra3 knockout mice do not show seizures (486). The mouse
line carrying the human GABRG2 p.Q390X DEE mutation, which has a dominant negative effect,
show a more severe phenotype compared to Gabrg2^{-/-} mice (487). Mutations in GABBR2, that
induce LoF with reduced slow GABAergic inhibition (488), have been identified in DEE (11, 16).

2.2 Synaptic transporters

DEE variants have also been identified in genes that although not directly implicated in synaptic
transmission are involved in synaptic functions.

SLC12A5 AND CLCN4. Cl^- is essential for numerous physiological functions, including GABAergic
inhibition. It is actively transported, and its concentration tightly regulated in neurons and virtually
all cells types. Cation-chloride co-transporters are postsynaptic plasma membrane proteins that
determine the intracellular Cl^- homeostasis and are thereby directly implicated in GABA_{A} current
generation (489). The gene family includes Na^+-Cl^- cotransporter (NCC), the Na^+-K^-2Cl^-
cotransporters (NKCC) and the K\(^{+}\)-Cl\(^{-}\) cotransporters (KCC). The \textit{SLC12A5} gene, exclusively expressed in the central nervous system, encodes the neuronal KCC2 K\(^{+}\)-Cl\(^{-}\) cotransporter, which is the major extruder of intracellular chloride in mature neurons. Low KCC2 activity can lead to increased intracellular Cl\(^{-}\) and to depolarizing GABAergic transmission (490).

\textit{SLC12A5} recessive \textit{de novo} mutations have been identified in a spectrum of epileptic disorders (491, 492). The most severe phenotype is a DEE with features of epilepsy of infancy with migrating focal seizures (EIMFS) caused by LoF mutations that decrease KCC2 surface expression and reduce protein glycosylation (139).

The \textit{CLCN} gene family contains nine members in mammals, four of which encode plasma membrane chloride channels (CLCN1, CLCN2, CLCNKA, CLCNKB) and five intracellular 2Cl\(^{-}\)/H\(^{+}\) exchangers (CLCN3–7) (493). Their function is not completely clear, but CLCN Cl\(^{-}\) channels are involved in the regulation of excitability by controlling extra- and intracellular ion homeostasis.

Dysfunction of some \textit{CLCN} genes leads to severe neurological disorders, in particular LoF mutations in \textit{CLCN4} cause a spectrum of phenotypes including severe DEE with drug-resistant seizures, cognitive and behavioral disorders (494, 495).

\textit{SLC1A2} AND \textit{SLC6A1}. \textit{SLC1A} are plasma membrane glutamate transporters expressed by glial cells and/or glutamatergic presynaptic terminals. They are essential for the removal and termination of action of glutamate released from the synapses. Mutations in \textit{SLC1A2}, encoding the astrocytic EAAT2 glutamate transporter selectively expressed in astrocytes, have been identified in DEE patients (496). Functional studies in transfected cell lines showed LoF and negative dominance (496), consistent with impaired clearance of extracellular glutamate. \textit{Slc1a2} knockout mice show a severe epilepsy phenotype only in homozygosity (497).

The \textit{SLC6A1} gene, encoding the GAT1 GABA transporter, is responsible for the reuptake of GABA into presynaptic terminals and astrocytes, are emerging as a common cause of DEE (498, 499).
Functional studies have shown that \textit{SLC6A1} DEE mutations induce LoF of \textit{GAT1} possibly associated with negative dominance and reduced GABA reuptake (481). \textit{Slc6a1}\textsuperscript{−/−} mice recapitulate some features of the human phenotypes, including motor and cognitive impairment, whereas heterozygous \textit{Slc6a1}\textsuperscript{+/−} mice do not show an overt phenotype (498). Overall, the mechanism may be similar to that of \textit{GABRD} mutations, which increase the extrasynaptic GABA\textsubscript{A} current (480).

\textbf{2.3 Other postsynaptic proteins}

Other mutations in genes encoding for receptor-associated proteins, such as the GTPase \textit{SynGAP1} (500) and Stargazin (501) are also associated with dysfunctions in circuit excitability and epileptic encephalopathies. Mutations in trans-synaptic adhesion protein genes, including neurexin-1, ILRAPL1, and Caspr2 were also reported to cause epilepsy in a limited number of cases (502–504).

\textbf{2.4 Extracellular synaptic proteins}

An interesting group of epilepsy-related genes encodes for proteins that are secreted at the synaptic cleft and contribute to trans-synaptic communication and synapse maintenance. Loss-of-function mutations in \textit{LGI1} gene (or LGI1 neutralizing autoantibodies) are associated with epilepsy with cognitive impairment (505). LGI1 is a protein secreted by both the presynaptic and postsynaptic neurons that is part of a trans-synaptic structural/functional bridge linking the integral proteins ADAM22 and ADAM23, exposed on the opposite sides of the synaptic cleft, that regulates the assembly and organization of AMPA glutamate receptors by the scaffold protein PSD95 (\textbf{FIGURE 8}) (140, 506). Other genes encoding for the synaptically secreted proteins SRPX2 and Reelin can cause epilepsy if mutated. These proteins play a role in synaptogenesis and in the neuronal development of the cerebral cortex by acting as extracellular synaptic organizers (507–510). In case of Reelin, the mutation impairs reelin secretion and the reelin variant is retained and eventually degraded through the autophagic way.

\textbf{IX. DYSFUNCTIONS IN NEURONAL HOUSEKEEPING: mTOR AND AUTOPHAGY}
Mature neurons are post-mitotic cells that do not replicate and have a very high workload throughout life. The housekeeping of their structure/function is therefore of paramount importance. Autophagy, a highly conserved structural turnover process that directs dysfunctional macromolecules and organelles to lysosomal degradation is an indispensable mean for neurons to maintain their integrity and functionality over time (511–513). Besides neuronal survival, autophagy plays a key role in neural development and in the formation and maintenance of synaptic connectivity. It is therefore conceivable that dysfunctional autophagy, due to mutations in genes controlling its multi-step processes, can cause severe neurodevelopmental disorders including DEEs.

One of the main pathways controlling autophagy and cell homeostasis is the mTOR (Mammalian/mechanistic target of rapamycin) pathway. mTOR is an atypical serine/threonine protein kinase that is activated by a very complex pathway integrating intra- and extra-cellular signals and strictly controlling matter and energy balance within the cell. The most important mTOR effectors are the p70 ribosomal S6 protein kinase-1 (p70S6K1) and the eukaryotic initiation factor 4E-binding proteins (4E-BPs) that transduce extracellular signals, such as neurotransmitters, growth factors, hormones, into an activation of translation. In neurons, these targets implicate mTOR in cell growth, neurite outgrowth and synaptic formation, all fundamental activities for neuronal functions and plasticity. The final synergistic effectors mTOR cascade are two mTOR complexes, mTORC1, mainly implicated in cell growth and proliferation, and mTORC2, mainly regulating cytoskeleton and dendrite growth (514, 515). Here we will focus on mTORC1, since virtually all pathogenic mutations identified thus far affect the mTORC1 cascade, and the implication of mTORC2 in DEEs is still limited. The activation of mTORC1 is tightly controlled by two inhibitory switches, the Tuberous Sclerosis Complex (TSC) and the GATOR1 complex (FIGURE 9). Both complexes are under the inhibitory control of two upstream complexes, GATOR2 that
inhibits GATOR1 and the phosphoinositide-3 kinase (PI3K)/Akt pathway that inhibits TSC. The canonical mTOR pathway starts with the activation of the PI3K/Akt pathway in response to extracellular signals (that is subjected to a further inhibitory control by PTEN) that relieves the mTOR complex from the tonic TSC inhibition. To be active, mTOR needs to attach to intracellular organelles, which requires the G-proteins Rheb and Rag (part of the Ragulator complex) to be in an active, GTP-bound state. The Ragulator complex, present on the membrane through an interaction with the proton pump vATPase, is a guanine nucleotide exchange factor (GEF) that activates Rag and allows mTOR membrane binding and activation. On the other hand, TSC and GATOR1 inhibitory complexes act as GTPase activating proteins (GAP) to deactivate Rheb and Rag, respectively, and thereby block mTOR activation. In addition to the stimulation of protein synthesis, activated mTOR inhibits autophagy by inhibiting the ULK1 complex that, through the sequential activation of the Beclin complex and the WD-repeat phosphoinositide-interacting protein 4 (WIPI4), activate the maturation and fusion of autophagosomes with acidic lysosomes to form autolysosomes (512). A central role in the regulation of mTOR activation, as well as in the autophagy progression, is played by the proton pump vATPase and its ancillary proteins that allows docking of Ragulator to the organelle membrane which in turn recruits and activates the mTORC1 complex (FIGURE 9).

Not surprisingly, dysregulation, particularly hyperactivation, of the mTOR pathway caused by mutations in the various components of the PI3K-mTOR pathway and regulatory cascades, result in DEEs, the so-called mTORopathies, that are often associated with malformations of cortical development. mTORopathies include a spectrum of drug-resistant epilepsy syndromes ranging from apparently non-lesional focal epilepsy to Tuberous Sclerosis Complex (TSC), FCDII, HME that are associated with brain malformations (515, 516). The complexity of the architecture of the mTOR pathway, while it guarantees a full control over key processes for cell survival and
adaptation, also offers several genes whose mutations impact on critical neuronal processes and are causative of DEEs (517). The first identified, prototypical mTORopathy is the TSC linked to germline/somatic mutations in TSC1 or TSC2 genes that remove the inhibitory brake of TSC on the downstream mTOR complex 1 (mTORC1) and determine mTOR hyperactivity in several tissues including the brain. Subsequently, high throughput sequencing has discovered an array of germline and de novo somatic mutations causing mTOR hyperactivity that involved either loss-of-function of inhibitory complexes (PI3K, PTEN, AKT3, TSC1, TSC2, RHEB, DEPDC5, NPRL2, and NPRL3) or gain-of-function hyperactive variants of MTOR. Animal models of mTORopathies are constantly characterized by an epileptic phenotype. Pten, Tsc1, Tsc2 and Depdc5 knockout mice, as well as Mtor knock-in mice recapitulate the human phenotype of mTORopathies by displaying dysplastic cortical areas with enlarged cortex, ectopic and hypertrophic neurons, and epilepsy. Constitutive and generalized mTOR hyperactivity is embryonic lethal, so that conditional models have been used to effectively reproduce human pathology. In addition to the dysplastic phenotype, mTOR hyperactivity causes hyperexcitability (515, 518, 519). This is not only attributable to the intrinsic instability of dysplastic circuits with dendritic and somatic hypertrophy and formation of reverberant connections, but also depends on an augmented intrinsic excitability of principal cortical neurons and potentiation of excitatory synaptic transmission at both pre- and post-synaptic levels promoting E/I imbalance. Another interesting feature of mTORopathies regards the relative frequency of somatic mutations that are likely to occur predominantly during brain development and that establish a condition of mosaicism in the neuronal populations of the cerebral cortex. The intersection between inherited germline mutations and acquired somatic ones ("second hit”) causes loss of heterozygosity or mosaic compound heterozygosity that can convert a latent deficit into an overt phenotype in restricted brain areas. This phenomenon was clearly demonstrated for DEPDC5 mutations both in humans and in experimental models in which
the “somatic” knockdown of Depdc5 was reproduced by in utero electroporation and/or RNA interference, generating focal cortical dysplasia (29, 520, 521). One of the mechanisms by which mTOR hyperactivity causes epileptic encephalopathies is through a dysregulation of autophagy, a process strongly implicated in neuronal survival and plasticity (511, 512, 520). As mentioned above, neuronal autophagy is a highly regulated process in which mTOR inhibition releases the ULK1 activator which, together with AMPK, activates the autophagy initiator Beclin complex (composed of VPS34, VPS15, Ambra1 and Beclin1), that is recruited to the phagophore, stimulates the production of PI3P and the binding of WIPI proteins. This allows the conversion of LC3-I into phosphatidylethanolamine-bound LC3-II that is the fundamental signal activating autophagy (FIGURE 9). mTOR and AMPK represent a yin-yang mechanism controlling autophagy: not only the two pathways exert an opposite control onto the underlying autophagic process, but AMPK also stimulates the TSC complex, keeping mTOR inactive during autophagy activation (522). The final common pathway of autophagy is the fusion of mature autophagosomes with lysosomes to form the autolysosomes, where the vesicular content is finally degraded and released into the cytoplasm. This event is regulated by the autophagic proteins EPG5 and SNX14 and requires internal acidification of the organelle to allow for proteolysis. Biallelic mutations of EPG5 and SNX14 cause respectively the Vici syndrome, a rare and severe congenital multisystem disorder characterized by failure of the corpus callosum, cataracts, oculocutaneous hypopigmentation, cardiomyopathy and combined immunodeficiency (523) and a form of cerebellar ataxia and intellectual disability (524). Acidification is provided by vATPase, a multifunctional proton pump that regulates multiple cellular processes including membrane trafficking, receptor-mediated endocytosis, SV cycling and NT loading. Acidification is necessary for autophagy progression and drugs or vATPase variants impairing the build-up of the proton gradient block autophagy (525). vATPase is composed of a V1 cytosolic domain that hydrolyses ATP and a V0 transmembrane
domain that transfers protons to the organelle’s interior (526). While the proton pumping activity of vATPase occurs in all tissues, the brain is particularly vulnerable to vATPase defects for its multiple functions in exocytosis (426, 527) and autophagy. In man, as many as 22 genes encode for the multiple and redundant subunits of the V1 and V2 complexes, allowing the composition of different v-ATPase complexes with specific properties and tissue expression.

Several, heterozygous or biallelic mutations in ATP6V1A coding for the V1 subunit A have been recently described in patients with DEEs of variable severity, ranging from moderate intellectual disability with seizures to an early-onset DEE with premature lethality (429, 528). ATP6V1A variants affect lysosomal homeostasis and autophagy and, when expressed in postsynaptic neurons, impair neurite development and the formation/maintenance of excitatory synaptic connections (429). ATP6V1A deficits have also been recently associated with neuronal impairment and neurodegeneration. ATP6V1A silenced neurons show reduced network activity and alteration of synaptic proteins consistent with a key role of ATP6V1A in neuronal maturation and activity (529).

In addition, mutations in ancillary proteins of the v-ATPase complex, known to regulate its function and trafficking to the organelles, also cause severe neurodevelopmental disorders with epilepsy and impair brain development when modelled in mice (430, 530). A de novo variant of ATP6AP2, a vATPase accessory protein, was identified in a patient with neurodevelopmental disorder and fulminant degeneration. In murine models and patient neurons, loss-of-function of ATP6AP2 results in lysosomal and autophagic defects with impaired neuronal survival, revealing a key role of this V-ATPase modulator in brain function (530). Homozygous recessive and compound heterozygous mutations in the DMXL2 gene were recently found to cause a severe and rapidly progressing DEE associated with Ohtahara syndrome and premature death (431). The gene encodes for the vesicular protein DMXL2 (also known as rabconnectin-3a), a member of the WD40
repeat (WDR) protein family that is highly expressed in brain tissue, regulates the trafficking and activity of V-ATPase and interacts with the SV-associated G-protein Rab3A (531, 532). Altered lysosomal homeostasis and defective autophagy were recapitulated in Dmlx2-silenced mouse hippocampal neurons that exhibit impaired neurite elongation and synaptic loss (431). Dmlx2⁻/⁻ mice are embryonic lethal (533) while heterozygous Dmlx2 mice show brain malformations, uncovering a penetrant role of Dmlx2 in brain development (534).

These data confirm a primary role of autophagy dysregulation in alterations of cortical development and epileptogenesis and suggest that the severity of the clinical phenotypes and the extent of neurodegeneration depend on the stage of neuronal development and on the specific consequences of the impairment of synaptic transmission and neuronal survival due to the stressful condition of impaired autophagy (535).

X. THE SEARCH FOR PERSONALISED TREATMENT APPROACHES

Currently, most therapies for DEEs target individual symptoms such as seizures, and not the underlying disease mechanisms. For many individuals with DEEs, seizure control is not achieved, even when antiseizure medications (ASMs) are optimized for the underlying etiology, while in patients in whom seizure control is achieved, developmental impairments and other comorbidities often remain severe (4, 397). In addition, since patients with the same type of clinical seizures may differently respond to ASMs, the pathophysiological events that underlie epileptic seizures apparently not only differ between unique seizure syndromes and specific etiologies, but may also be multifactorial for the same types (536). The remarkable growth of animal models of DEEs has enabled preclinical studies that tested several experimental drugs have been tested in different animal models of DEEs (TABLE 4). Both acute and chronic models of epileptic spasms have been used to study effects on spasms prevention using protocols that administer drugs prior to the induction (acute models and certain chronic models) (537–544), spasms control / cessation
(chronic models: multiple-hit, TTX) (150, 155–158, 160, 161, 545), hypsarrhythmia (TTX model) (546), disease modification or antiepileptogenesis (chronic models: multiple-hit, ARX KI) (150, 155). Among the existing models, resistance to existing therapies for ISS (ACTH, vigabatrin) is shown at the multiple-hit rat model of ISS due to structural lesions (88). The only model that has been used to study effects on hypsarrhythmia is the TTX model which allows testing of effects on the EEG using multiple electrodes in post-pubertal animals. This is technically challenging in rodent models that manifest spasms only during restricted early developmental periods, when the skull is small and fragile. The prenatal betamethasone/postnatal NMDA model suggested that prenatal betamethasone increases the sensitivity to ACTH, even though in increases the severity of NMDA-induced spasms and has been used to explore pharmacologically therapeutic effects of pathways thought to mediate the ACTH effects, i.e., mineralocorticoid receptor signaling (538). Among the treatments tested in these preclinical trials, two drugs eventually acquired orphan status for ISS (carisbamate, CPP-115). CPP-115 has also been tested in a case report on an infant with drug-resistant ISS with significant improvement in spasms and seizure control compared to vigabatrin (547). In support of the promising effects of the mTOR inhibitor rapamycin on spasms in the multiple-hit model, recent clinical case reports suggested a possible benefit in infants with tuberous sclerosis related ISS treated with the mTOR inhibitor everolimus (548).

There has also been a remarkable progress in screening for new treatments for Dravet syndrome, using both rodents (233, 384, 549–558) as well as high throughp ut studies in zebrafish (391, 559–564). A noteworthy development has been the recent approval by the Food and Drug Administration of fenfluramine, a reuptake inhibitor of 5-OH-tryptamine, for the treatment of Dravet syndrome, based on evidence provided by both preclinical (560, 561) and clinical trials (230, 565–569).
A challenge in these preclinical drug trials has been the comparison of findings across models, given the different species (rats vs other animal models), different induction protocols, developmental periods of exposure to the drugs, and treatment protocols (pre- vs post-treatments) (150, 155, 157, 161, 537, 542, 545). Hopefully identification of biomarkers guiding treatment implementation would help de-risk the process of selecting promising candidates for transitioning in clinical trials, as well as optimizing treatment protocols and designs.

The choice of ASM for DEE in clinical practice is mainly influenced by cumulative experience in open label studies and only a limited number of drug trials in etiologically homogeneous conditions (570). For most DEE, treatment choices rely on the hypothesized drug action on clinical and EEG phenomenology and remain confined to symptom relief and general principles of epilepsy management. Commercially available ASMs are typically grouped by their principal mode of action (drugs that affect voltage-dependent sodium channels, calcium currents, GABA activity, the glutamate receptors, and drugs with other mechanisms of action - TABLE 5), although for many drugs the precise mechanism of action remains unknown, or multiple actions are hypothesized. As for other conditions, treatments for DEEs may include conventional drugs or repurposed therapies (i.e., with specific actions that may have been used in entirely unrelated conditions).

Only recently, the enormous amount of knowledge generated by molecular genetic findings in the DEE and increased knowledge of the underlying disease mechanisms has allowed designing etiology-specific trials for genetic DEEs (https://clinicaltrials.gov/ct2/home), (see TABLE 6 and SUPPLEMENTAL TABLE S2, https://figshare.com/articles/dataset/Guerrini_et_al_Supplemental_Table_S2_xlsx/17694728, for examples).

When clinical, EEG, and imaging findings suggest focal localization of the epileptogenic zone, and if any resulting neurologic deficit is not more severe than epilepsy itself, surgical treatment of
epilepsy remains one of the best options. For example, for patients with TSC, epilepsy surgery is considered after failure of two ASMs even when multiple cortical lesions are present and TSC operated patients, including those with infantile spasm, have a 50%–60% chance of long-term seizure freedom after surgery for epilepsy (570).

A promising alternative approach to treat DEEs is represented by gene therapies. The growth in gene therapies in medicine has partly been realized through the development of safe and effective means of gene delivery using viral vectors. These vectors are engineered to avoid their replication in human cells and to deliver the wild type form of the gene mutated in a given patient into the right cell target. The expression of the gene product delivered by viral vectors in the cells where the genetic defect needs to be corrected is ensured using a specific promoter. For DEEs, this treatment approach has some drawbacks, as most viral vectors can only carry a limited amount of DNA (and thus cannot be used to replace big genes such as \textit{SCN1A}). In addition, to bypass the blood brain barrier (BBB), they need to be injected in the brain through intraventricular injections. Gene therapy based on vectors derived from adeno-associated virus (AAV) has the potential to overcome the latter limitation since it has been demonstrated that different AAV serotypes are able to cross the BBB and thus can be used to implement gene delivery strategies based on them to treat CNS diseases (571). Gene therapy, including AAV for DEE carries several major technical limitations (572). Delivery would need to target the whole brain as most DEE are related to widespread brain dysfunction. Diffusely and irreversibly altering the genetic make-up of neurons may be a worrisome perspective since some of the genetic defects causing DEE result from both loss and gain of function effects an overdosing can cause its own pathology. Dosing and distribution are further complicated by the X-linked or somatic mosaic genetic alteration that underlies the disorder, with only half or small percentages of cells needing the supplemental
transgene delivery. Additional limitations are related to the fact that delivery should be realized in a critical period, before mature networks are established.

Another possible way to correct the effects of a mutation at intracellular level using genetic material is the use of antisense oligonucleotides (ASOs), single stranded deoxyribonucleotides which are complementary to a specific target mRNA and that, upon binding it alters its splicing, impede its translation, or promote its degradation. ASOs are usually administered intrathecally. Studies on a conditional mouse model with Cre-dependent expression of the pathogenic patient SCN8A mutation p.Arg1872Trp (R1872W) have recently demonstrated that administration of an ASO directed against the Scn8a mutant transcript delayed seizure onset and increased survival, suggesting that reduction of SCN8A transcript is a promising approach to treatment of intractable childhood epilepsies (262). The effectiveness of ASOs has been confirmed in an additional study carried out by Li and collaborators (246). These authors demonstrated that targeted reduction of Scn2a mRNA expression by central administration of a gapmer ASOs in Scn2a Q/+ mice reduced spontaneous seizures and significantly extended life span in treated animals. These results suggest that human SCN2A gapmer ASOs could likewise impact the lives of patients with SCN2A gain-of-function DEE (246). Because ASOs can regulate splicing, it is possible to use them to increase the production of translated mRNA. TANGO (targeted augmentation of nuclear gene output) approach, which modulates naturally occurring, nonproductive splicing events to increase target gene and protein expression, has recently been used in animal models of DS to increase Scn1a transcript and Na\textsubscript{v}1.1 protein expression. In these models, a single intracerebroventricular dose of a lead ASO at postnatal day 2 or 14 reduced the incidence of electrographic seizures and sudden unexpected death in epilepsy (SUDEP), suggesting that TANGO may provide a unique, gene-specific approach for the treatment of DS (233).
ASOs can also be used to regulate the activity of miRNAs, which are ‘multi-pathway’ regulatory molecules. Mature miRNAs are generated via a multistep process. They are initially transcribed as relatively large (even more than 1 kb) hairpin structures known as pri-miRNA. This undergoes cleavage in the nucleus by the enzyme Drosha to produce a 60–70-nucleotide (nt) stem loop pre-miRNA, which is subsequently transported from the nucleus to the cytoplasm. The enzyme Dicer recognizes pre-miRNA and cleaves the stem loop, leaving an imperfect ~21–23-nt miRNA duplex with a ~2-nt 3’ overhang at each end. The less thermodynamically stable end of the pre-miRNA duplex is then uploaded to a binding pocket within an Argonaute (Ago) protein to form the RNA-induced silencing complex (RISC). The miRNA-loaded RISC then traffics along mRNAs searching for complementary binding sites and, upon finding mRNA targets containing a ~7–8-nt seed match [typically within the 3’ untranslated region (UTR)], triggers either target degradation or translational repression (573). Since there have been more than 300 studies on miRNA and epilepsy, and over 100 different miRNAs have found to be altered in experimental models and human samples [EpimiRBase (574)], miRNA could represent a novel class of molecules to be targeted using ASOs to treat epilepsy in DEEs.

Gene therapy can be also pursued correcting a specific genetic defect in patients’ cells through endogenous gene editing. This approach is not based on expressing the wild type form of a given gene in cells that do not express it properly, but on the substitution of a specific portion of the endogenous mutated gene (e.g. that flanking a mutation) with the wild type sequence. Clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 is a gene-editing technology that makes it possible to correct errors in the genome and turn on or off genes in cells and organisms quickly, cheaply and with relative ease. This gene-editing technology has a number of laboratory applications including rapid generation of cellular and animal models, functional genomic screens, live imaging of the cellular genome, and gene therapy (575, 576). CRISPR/Cas9 involves two
essential components: a guide RNA to match a desired target gene, and Cas9 (CRISPR-associated protein 9) - an endonuclease which causes a double-stranded DNA break, allowing modifications of the genome. One of the most exciting applications of CRISPR/Cas9 is its potential use to treat genetic disorders caused by single gene mutations. Examples of such diseases include cystic fibrosis (CF), Duchenne’s muscular dystrophy (DMD) and haemoglobinopathies (576). A modified version of the CRISPR-Cas9 system, called dead Cas9 (dCas9), can be tailored to obtain a robust and highly specific activation of the \textit{Scn1a} gene both in cultured neurons and in the brain tissue of a DS mouse model, suggesting that dCas9 may be an effective and targeted approach to DS and other disorders resulting from altered gene dosage (232). A number of challenges remain before the potential of CRISPR/Cas9 can be translated to effective treatments at the bedside. Indeed, its clinical translation has been hampered by varying efficiency, off-target effects, and, on occasion, insufficient vector size for the necessary genetic material (570). In addition, a suitable vector is needed to safely deliver Cas9-nuclease encoding genes and guide RNAs \textit{in vivo} without any associated toxicity (576). To overcome this problem, the use of AAV vectors has been proposed. However, this delivery system may be too small to allow efficient transduction of the Cas9 gene. A smaller Cas9 gene could be used, but this has additional implications on efficacy (575).

\textbf{XI. CONCLUSIONS}

Multiple genetically determined or, at times, acquired etiologies may severely alter the balance between excitatory and inhibitory neuronal activity and result in widespread epileptogenesis in the developing brain. If the causative defect imposes serious consequences on physiological brain function, the superimposed epilepsy will add a clinical burden to the already compromised neurodevelopmental processes. If the causative defect only mildly impacts physiological brain development and function, any superimposed severe epileptogenic process will cause considerable neurodevelopmental deterioration (577). Development of higher cortical functions is
the most complex and vulnerable process and will be the most severely affected. Impairment may be relatively selective, in the context of DEE in which for example, language, memory, attention or executive functions are impaired to a different extent. Generalized severe cognitive impairment or autistic features may be the result of a widespread epileptogenic process. Although DEE have a multitude of causes and variable clinical patterns, with many genes involved whose altered expression may affect different aspects of neural cell functioning, mechanisms whereby epileptic activity may interfere with brain function and produce such patterns, tend to be relatively limited. Moderate levels of hyperexcitability can disrupt cortical processing, with temporal and anatomic specificity (578). Disruption is time-locked with the EEG event accompanying hyperexcitability and specific to the modality represented in the anatomic area involved (579–581). Redundant and frequent EEG abnormalities as observed in DEE may cause widespread cortical dysfunction that can be manifested with signs of early cognitive regression even when seizure activity is not yet overly manifested (582, 583). The timing of onset, network distribution and duration of the epileptogenic process influence how the DEE will be manifested, at times in close relationship with its specific etiology, at times irrespective from it similar anatomicclinical backgrounds may be accompanied by very different forms of DEE in different individuals. In this perspective, while efforts towards development of precision treatment approaches in DEE shall ideally target the causative mechanisms, traditional approaches with antiseizure medications addressed at mitigating the consequences of redundant epileptic activity on physiological brain function still have a relevant role.

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**Table 1. Developmental and epileptic encephalopathies (DEEs) by age**

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<th>Age of onset</th>
<th>Gender</th>
<th>Etiology</th>
<th>Electroclinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neonatal – Infantile onset</strong></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
| Early infantile DEEs (previously Ohtahara syndrome or early myoclonic epilepsy) | ≤3mo of life | M=F | Genetic or structural/metabolic | **Clinical**: Abnormal neurological findings, neurodevelopmental deficits. Seizures: tonic (independent of sleep), myoclonic, epileptic spasms, sequential.  
**EEG**: Burst-suppression pattern; multifocal epileptiform discharges; hypsarrhythmia may appear. Seizure patterns are bilateral or focal onset, depending on seizure types. |
| Epilepsy of Infancy with Migrating Focal Seizures (EIMFS) | First year of life | M=F | Mainly genetic | **Clinical**: Neurodevelopmental delay, focal motor tonic or clonic seizures.  
**EEG**: Migrating EEG patterns during ictal events, multifocal discharges |
| Infantile spasms syndrome (ISS) | 3-12mo (1-24mo) | M>F | Structural/metabolic, genetic, unknown | **Clinical**: Epileptic spasms, other seizures may occur; neurodevelopmental disorders, intellectual disabilities.  
**EEG**: Hypsarrhythmia, electrodecremental responses (ictal or interictal), multifocal epileptiform discharges. |
<p>| Dravet syndrome | 3-9mo (1-20mo) | M=F | Genetic | <strong>Clinical</strong>: Neurodevelopmental deficits. Prolonged hemiclonic seizure with fever in the absence of infectious / structural brain lesion; myoclonic, focal impaired awareness, atypical absences, atonic seizures, non-convulsive status epilepticus, tonic and tonic-clonic seizures in sleep. |</p>
<table>
<thead>
<tr>
<th>Age of onset</th>
<th>Gender</th>
<th>Etiology</th>
<th>Electroclinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>First year of life</td>
<td>M=F, M&lt;F</td>
<td>Genetic</td>
<td><strong>EEG</strong>: Focal or multifocal epileptiform abnormalities and seizures, photoparoxysmal responses. <strong>KCNQ2-DEE</strong>: Sequential or focal tonic seizures, burst suppression; autonomic symptoms, epileptic spasms. Burst suppression or multifocal EEG. <strong>CDK5L5-DEE</strong>: Tonic seizures, epileptic spasms. hypermotor-tonic-spasms; movement disorders <strong>PDE, PSPDE</strong>: Intrauterine or early life seizures; focal seizures, spasms or generalized tonic-clonic; response to pyridoxine or PSP. EEG: burst suppression or multifocal discharges. <strong>Glut1DS</strong>: Intellectual disability, low CSF/plasma glucose ratio, generalized seizures (myoclonic, myoclonic-atonic, generalized tonic-clonic, absences). EEG: 2.5-5Hz spike-wave. <strong>PCDH19-DEE</strong>: Intellectual disability, autism spectrum disorder; focal impaired aware to tonic or atypical absence seizures. EEG: focal onset seizures. <strong>Sturge-Weber</strong>: Facial port-wine stain, progressive neurological course, epilepsy, hemiparesis, psychomotor delay, stroke-like events, psychiatric disorders, glaucoma. Focal motor seizures, febrile seizures, infantile spasms, myoclonic-atonic, gelastic seizures. EEG: Asymmetric, focal epileptiform activities.</td>
</tr>
</tbody>
</table>

**Etiology-specific syndromes:**
- **KCNQ2, CDKL5, PCDH19, SCL2A1, Pyridoxine and Pyridox(am)ine 5’-Phosphate Dependent Epilepsy**
- **Glucose Transporter 1 Deficiency syndrome (Glu1DS)**
- **Sturge-Weber syndrome**
<table>
<thead>
<tr>
<th>Condition</th>
<th>Age of onset</th>
<th>Gender</th>
<th>Etiology</th>
<th>Electroclinical characteristics</th>
</tr>
</thead>
</table>
| Gelastic seizures with Hypothalamic Hamartoma | First year of life | M=F    | Structural, Genetic-structural  | *Clinical*: Normal neurological exam initially, deficits appear later; precocious puberty; gelastic seizures with mirthless laughter (mandatory), gelastic and dacrystic seizures, focal impaired awareness or generalized seizures, other types of seizures may occur.  
*EEG*: Focal or generalized |

**Childhood onset**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Age of onset</th>
<th>Gender</th>
<th>Etiology</th>
<th>Electroclinical characteristics</th>
</tr>
</thead>
</table>
| Myoclonic-atonic epilepsy                      | 2-6y         | M>F    | Genetic                         | *Clinical*: Seizures: myoclonic-atonic (mandatory), atonic, myoclonic, absence, generalized tonic-clonic.  
*EEG*: 3-6Hz (poly)spike-slow wave discharges, generalized, activated in sleep; generalized paroxysmal fast |
| Lennox-Gastaut syndrome                        | 18mo – 8y    | (M>F)  | Structural/metabolic, genetic   | *Clinical*: Tonic seizures in sleep (mandatory), atypical absence, atonic, myoclonic, focal impaired awareness, generalized tonic-clonic, epileptic spasms  
*EEG*: Slow spike-wave (≤2.5Hz), generalized; generalized paroxysmal fast; focal or multifocal slow spike-wave may be seen. |
| DEE with SW activation in sleep (D/EE-SWAS)     | 2-12y        | M=F    | Structural, genetic             | *Clinical*: Neurocognitive / behavioral deficits which ameliorate / resolve with resolution of SWAS; seizure types depend on etiology: focal or focal to bilateral, typical, or atypical absences, atonic, negative myoclonus.  
*EEG*: Slow background; focal or multifocal discharges; marked activation of diffuse epileptiform discharges in sleep (>50% of sleep, 1.5-2Hz spike-wave runs). |
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Age of onset</th>
<th>Gender</th>
<th>Etiology</th>
<th>Electroclinical characteristics</th>
</tr>
</thead>
</table>
| **Febrile Infection Related Epilepsy Syndrome (FIRES)** | 2-17         | (M>F)  | Infectious/post-infectious | *Clinical*: Developmental regression, intellectual disabilities, attention or behavioral problems, motor dysfunction. Focal or multifocal seizures, super-refractory status epilepticus.  
*EEG*: Slow background, multifocal epileptiform discharges; extreme delta brushes. Increasing frequency of focal onset seizures (focal >10Hz evolving to rhythmic spike-waves). |
| **Hemiconvulsion-Hemiplegia-Epilepsy syndrome (HHE)** | <4y          | M=F    | Unknown, structural/metabolic, genetic | *Clinical*: Focal (clonic) febrile status epilepticus and persistent hemiparesis, aphasia when dominant hemisphere involved; focal or focal to bilateral motor seizures.  
*EEG*: Focal or lateralized rhythmic ictal delta, focal recruiting (10Hz) rhythms.                                                                                                                                 |
| **Progressive myoclonus epilepsy (PME)**           |              |        | Genetic                 |                                                                                                                                                                                                                                  |
| - **Unverricht-Lundborg (EPM1)**                   | 7-13y        | M=F    | Genetic                 | *Clinical*: progressive course; myoclonus induced by touch / photic stimulation, more pronounced upon awakening; other generalized seizures may occur.  
*EEG*: Photosensitivity; generalized polyspike and wave (ictal).                                                                                                                                                             |
*EEG*: Photosensitivity; spike wave and polyspikes, no activation in sleep.                                                                                                                                                 |
<table>
<thead>
<tr>
<th>Age of onset</th>
<th>Gender</th>
<th>Etiology</th>
<th>Electroclinical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neuronal Ceroid Lipofuscinosis (NCL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- <strong>CLN2 (late infantile)</strong></td>
<td>2-4yo</td>
<td>M=F</td>
<td>Genetic</td>
</tr>
<tr>
<td>Clinical: Language delay, progressive course, multiple seizures febrile and afebrile, including myoclonic.</td>
<td></td>
<td></td>
<td>EEG: Photoparoxysmal response at low frequencies.</td>
</tr>
<tr>
<td>EEG:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- <strong>Juvenile CLN3</strong></td>
<td>4-10y</td>
<td>M=F</td>
<td>Genetic</td>
</tr>
<tr>
<td>Clinical: Visual loss progressive, macular degeneration, optic atrophy, retinitis pigmentosa.</td>
<td></td>
<td></td>
<td>EEG:</td>
</tr>
<tr>
<td>EEG:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- <strong>Adult NCL (Kufs)</strong></td>
<td>11-50y</td>
<td>M=F</td>
<td>Genetic</td>
</tr>
<tr>
<td>Type A: PME with dementia and ataxia.</td>
<td></td>
<td></td>
<td>Type B: Dementia with cerebellar or extrapyramidal motor symptoms but not PME.</td>
</tr>
<tr>
<td>Type B: Dementia with cerebellar or extrapyramidal motor symptoms but not PME.</td>
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</tr>
</tbody>
</table>

The list of DEEs follows the 2021 proposal of the International League Against Epilepsy (ILAE) Task Force on Nosology and Definitions for epilepsy syndromes (5–7). DEE: Developmental and epileptic encephalopathies; D/EE-SWAS: DEE with SW activation in sleep; EIMFS: Epilepsy of Infancy with Migrating Focal Seizures; EPM: epilepsy with progressive myoclonus; F: female; Glut1DS: Glucose Transporter 1 (SLC2A1) Deficiency syndrome; HHE: Hemiconvulsion-Hemiplegia-Epilepsy syndrome; M: male; mo: months; NCL or CLN: neuronal ceroid lipofuscinosis; PME: progressive myoclonic epilepsy; PDE: Pyridoxine dependent epilepsy; P5PDE: Pyridox(am)ine 5'-Phosphate (P5PD) Dependent Epilepsy; SW: spike-and-slow-wave; y: years.
<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KCNA2</strong></td>
<td>Potassium channel, voltage-gated, Shaker-related subfamily, member 2</td>
</tr>
<tr>
<td><strong>CACNA1E</strong></td>
<td>Calcium channel, voltage-dependent, alpha 1E subunit</td>
</tr>
<tr>
<td><strong>KCNT2</strong></td>
<td>Potassium channel, subfamily T, member 2</td>
</tr>
<tr>
<td><strong>SCN3A</strong></td>
<td>Sodium channel, voltage-gated, type III, alpha polypeptide</td>
</tr>
<tr>
<td><strong>SCN2A</strong></td>
<td>Sodium channel, voltage-gated, type II, alpha subunit</td>
</tr>
<tr>
<td><strong>SCN1A</strong></td>
<td>Sodium channel, voltage-gated, type I, alpha polypeptide</td>
</tr>
<tr>
<td><strong>SCN9A</strong></td>
<td>Sodium channel, voltage-gated, type IX, alpha subunit</td>
</tr>
<tr>
<td><strong>CACNA2D2</strong></td>
<td>Calcium channel, voltage-dependent, alpha-2/delta subunit 2</td>
</tr>
<tr>
<td><strong>HCN1</strong></td>
<td>Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1</td>
</tr>
<tr>
<td><strong>KCNQ5</strong></td>
<td>Potassium channel, voltage-gated, KQT-like subfamily, member 5</td>
</tr>
<tr>
<td><strong>KCNT1</strong></td>
<td>Potassium channel, subfamily T, member 1</td>
</tr>
<tr>
<td><strong>CACNA1B</strong></td>
<td>Calcium channel, voltage-dependent, L type, alpha 1B subunit</td>
</tr>
<tr>
<td><strong>SCN8A</strong></td>
<td>Sodium channel, voltage gated, type VIII, alpha polypeptide</td>
</tr>
<tr>
<td><strong>CACNA1G</strong></td>
<td>Calcium channel, voltage-dependent, T type, alpha-1G subunit</td>
</tr>
<tr>
<td><strong>CACNA1A</strong></td>
<td>Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit</td>
</tr>
<tr>
<td><strong>SCN1B</strong></td>
<td>Sodium channel, voltage-gated, type I, beta polypeptide</td>
</tr>
<tr>
<td><strong>KCNB1</strong></td>
<td>Potassium voltage-gated channel, Shab-related subfamily, member 1</td>
</tr>
<tr>
<td><strong>KCNQ2</strong></td>
<td>Potassium voltage-gated channel, KQT-like subfamily, member 2</td>
</tr>
<tr>
<td><strong>CLCN4</strong></td>
<td>Chloride channel-4</td>
</tr>
<tr>
<td>Approved Symbol</td>
<td>Gene/Locus name</td>
</tr>
<tr>
<td>-----------------</td>
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</tr>
<tr>
<td><strong>Receptors</strong></td>
<td></td>
</tr>
<tr>
<td><strong>GABRA2</strong></td>
<td>Gamma-aminobutyric acid (GABA) A receptor, alpha-2</td>
</tr>
<tr>
<td><strong>GABRB1</strong></td>
<td>Gamma-aminobutyric acid (GABA) A receptor, beta-1</td>
</tr>
<tr>
<td><strong>GABRB2</strong></td>
<td>Gamma-aminobutyric acid (GABA) A receptor, beta-2</td>
</tr>
<tr>
<td><strong>GABRA1</strong></td>
<td>Gamma-aminobutyric acid (GABA) A receptor, alpha-1</td>
</tr>
<tr>
<td><strong>GABRG2</strong></td>
<td>Gamma-aminobutyric acid (GABA) A receptor, gamma-2</td>
</tr>
<tr>
<td><strong>NTRK2</strong></td>
<td>Neurotrophic tyrosine kinase, receptor, type 2</td>
</tr>
<tr>
<td><strong>GABBR2</strong></td>
<td>Gamma-aminobutyric acid B receptor 2</td>
</tr>
<tr>
<td><strong>GRIN1</strong></td>
<td>Glutamate receptor, ionotropic, N-methyl D-aspartate 1</td>
</tr>
<tr>
<td><strong>GABRB3</strong></td>
<td>Gamma-aminobutyric acid (GABA) A receptor, beta-3</td>
</tr>
<tr>
<td><strong>GABRA5</strong></td>
<td>Gamma-aminobutyric acid (GABA) A receptor, alpha-5</td>
</tr>
<tr>
<td><strong>GRIN2A</strong></td>
<td>Glutamate receptor, ionotropic, N-methyl D-aspartate 2A</td>
</tr>
<tr>
<td><strong>GRIN2D</strong></td>
<td>Glutamate receptor, ionotropic, N-methyl-D-aspartate 2D</td>
</tr>
<tr>
<td><strong>Transporters</strong></td>
<td></td>
</tr>
<tr>
<td><strong>ATP1A2</strong></td>
<td>ATPase, Na+/K+ transporting, alpha-2 polypeptide</td>
</tr>
<tr>
<td><strong>SLC2A1</strong></td>
<td>Solute carrier family 2 (facilitated glucose transporter), member 1</td>
</tr>
<tr>
<td><strong>ARV1</strong></td>
<td>ARV1 homolog, fatty acid homeostasis modulator</td>
</tr>
<tr>
<td><strong>SLC1A4</strong></td>
<td>Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4</td>
</tr>
<tr>
<td><strong>SLC25A12</strong></td>
<td>Solute carrier family 25 (mitochondrial carrier, Aralar), member 12</td>
</tr>
<tr>
<td><strong>SLC6A1</strong></td>
<td>Solute carrier family 6 (neurotransmitter transporter, GABA), member 1</td>
</tr>
<tr>
<td><strong>SLC25A22</strong></td>
<td>Solute carrier family 25 (mitochondrial carrier, glutamate), member 22</td>
</tr>
<tr>
<td><strong>SLC1A2</strong></td>
<td>Solute carrier family 1 (glial high affinity glutamate transporter),</td>
</tr>
<tr>
<td>Approved Symbol</td>
<td>Gene/Locus name</td>
</tr>
<tr>
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<tr>
<td>SLC13A5</td>
<td>Solute carrier family 13 (sodium-dependent citrate transporter), member 5</td>
</tr>
<tr>
<td>SLC25A10</td>
<td>Solute carrier family 25 (mitochondrial carrier), member 10 (dicarboxylate ion carrier)</td>
</tr>
<tr>
<td>SLC25A42</td>
<td>Solute carrier family 25, member 42</td>
</tr>
<tr>
<td>ATP1A3</td>
<td>ATPase, Na+/K+ transporting, alpha-3 polypeptide</td>
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<tr>
<td>SLC12A5</td>
<td>Solute carrier family 12, (potassium-chloride transporter) member 5</td>
</tr>
<tr>
<td>SLC35A2</td>
<td>Solute carrier family 35 (UDP-galactose transporter), member 2</td>
</tr>
<tr>
<td>SLC9A6</td>
<td>Solute carrier family 9 (sodium/hydrogen exchanger), member 6</td>
</tr>
</tbody>
</table>

**Synapse related**

<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
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</thead>
<tbody>
<tr>
<td>CPLX1</td>
<td>Complexin 1</td>
</tr>
<tr>
<td>PPP3CA</td>
<td>Protein phosphatase 3, catalytic subunit, alpha isoform (calcineurin A alpha)</td>
</tr>
<tr>
<td>SYNGAP1</td>
<td>Synaptic Ras GTPase activating protein 1</td>
</tr>
<tr>
<td>ADAM22</td>
<td>ADAM metallopeptidase domain 22</td>
</tr>
<tr>
<td>STXBP1</td>
<td>Syntaxin-binding protein 1</td>
</tr>
<tr>
<td>DNM1</td>
<td>Dynamin-1</td>
</tr>
<tr>
<td>NECAP1</td>
<td>NECAP endocytosis-associated protein 1</td>
</tr>
<tr>
<td>DMXL2</td>
<td>DMX-like 2</td>
</tr>
<tr>
<td>AP3B2</td>
<td>Adaptor-related protein complex 3, beta 2 subunit</td>
</tr>
<tr>
<td>STX1B</td>
<td>Syntaxin 1B</td>
</tr>
<tr>
<td>SYNJ1</td>
<td>Synaptojanin 1</td>
</tr>
<tr>
<td>NRXN1</td>
<td>Neurexin 1</td>
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</table>

**Cell growth, division, and proliferation related**
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<th>Approved Symbol</th>
<th>Gene/Locus name</th>
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</thead>
<tbody>
<tr>
<td><strong>MTOR</strong></td>
<td>Mechanistic target of rapamycin</td>
</tr>
<tr>
<td><strong>AKT3</strong></td>
<td>AKT serine/threonine kinase 3</td>
</tr>
<tr>
<td><strong>NPRL2</strong></td>
<td>NPR2-like protein, GATOR1 complex subunit</td>
</tr>
<tr>
<td><strong>STAG1</strong></td>
<td>Stromal antigen 1</td>
</tr>
<tr>
<td><strong>RNF13</strong></td>
<td>RING finger protein 13</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>Phosphatidylinositol 3-kinase, catalytic, alpha</td>
</tr>
<tr>
<td><strong>PPP2CA</strong></td>
<td>Protein phosphatase-2 (formerly 2A), catalytic subunit, alpha isoform</td>
</tr>
<tr>
<td><strong>ACTL6B</strong></td>
<td>Actin-like 6B</td>
</tr>
<tr>
<td><strong>RHOBTB2</strong></td>
<td>Rho-related BTB domain-containing protein 2</td>
</tr>
<tr>
<td><strong>TSC1</strong></td>
<td>Hamartin</td>
</tr>
<tr>
<td><strong>AKT1</strong></td>
<td>AKT serine/threonine kinase 1</td>
</tr>
<tr>
<td><strong>NPRL3</strong></td>
<td>Nitrogen permease regulator-like 3</td>
</tr>
<tr>
<td><strong>TSC2</strong></td>
<td>Tuberin</td>
</tr>
<tr>
<td><strong>PIK3R2</strong></td>
<td>Phosphatidylinositol 3-kinase, regulatory subunit 2</td>
</tr>
<tr>
<td><strong>DEPDC5</strong></td>
<td>DEP domain-containing protein 5</td>
</tr>
</tbody>
</table>

**Cell metabolism related**

<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MTHFR</strong></td>
<td>Methylenetetrahydrofolate reductase</td>
</tr>
<tr>
<td><strong>ST3GAL3</strong></td>
<td>ST3 beta-galactoside alpha-2,3-sialyltransferase 3</td>
</tr>
<tr>
<td><strong>PARS2</strong></td>
<td>Prolyl-tRNA synthetase 2</td>
</tr>
<tr>
<td><strong>HNRNPU</strong></td>
<td>Heterogeneous nuclear ribonucleoprotein U</td>
</tr>
<tr>
<td><strong>CAD</strong></td>
<td>CAD trifunctional protein of pyrimidine biosynthesis</td>
</tr>
<tr>
<td><strong>MDH1</strong></td>
<td>Malate dehydrogenase, soluble</td>
</tr>
<tr>
<td><strong>UGP2</strong></td>
<td>Uridylic diphosphate glucose pyrophosphorylase-2</td>
</tr>
<tr>
<td><strong>BOLA3</strong></td>
<td>bolA family member 3</td>
</tr>
<tr>
<td><strong>ST3GAL5</strong></td>
<td>Sialyltransferase 9</td>
</tr>
<tr>
<td>Approved Symbol</td>
<td>Gene/Locus name</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>GAD1</td>
<td>Glutamate decarboxylase-1, brain, 67kD</td>
</tr>
<tr>
<td>GLS</td>
<td>Glutaminase</td>
</tr>
<tr>
<td>D2HGDH</td>
<td>D-2-hydroxyglutarate dehydrogenase</td>
</tr>
<tr>
<td>UGDH</td>
<td>UDP-glucose dehydrogenase</td>
</tr>
<tr>
<td>MANBA</td>
<td>Mannosidase, beta A, lysosomal</td>
</tr>
<tr>
<td>NUS1</td>
<td>NUS1 dehydrodolichyl diphosphate synthase subunit</td>
</tr>
<tr>
<td>MDH2</td>
<td>Malate dehydrogenase, mitochondrial</td>
</tr>
<tr>
<td>DENND5A</td>
<td>DENN domain-containing protein 5A</td>
</tr>
<tr>
<td>NARS2</td>
<td>Asparaginyl-tRNA synthetase 2</td>
</tr>
<tr>
<td>ALG9</td>
<td>ALG9 alpha-1,2-mannosyltransferase</td>
</tr>
<tr>
<td>FCSK</td>
<td>Fucose kinase</td>
</tr>
<tr>
<td>PNPO</td>
<td>Pyridoxamine 5’-phosphate oxidase</td>
</tr>
<tr>
<td>ITPA</td>
<td>Inosine triphosphatase-A</td>
</tr>
<tr>
<td>ALG13</td>
<td>ALG13 UDP-N-acetylglicosaminyltransferase subunit</td>
</tr>
</tbody>
</table>

**Intracellular trafficking related**

| TRAK1           | Trafficking protein, kinesin-binding 1 |
| AP2M1           | Adaptor-related protein complex 2, mu 1 subunit |
| CAMK2G          | Calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma |
| TBC1D24         | TBC1 domain family, member 24 |
| NSF             | N-ethylmaleimide-sensitive factor |
| CLTC            | Clathrin, heavy polypeptide (Hc) |
| ARX             | Aristaless-related homeobox, X-linked |

**Intracellular signaling related**

<p>| SZT2            | SZT2 subunit of KICSTOR complex |
| DOCK7           | Deducator of cytokinesis 7 |
| YWHAG           | Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase |</p>
<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>activation protein, gamma isoform</td>
</tr>
<tr>
<td><strong>GNAO1</strong></td>
<td>Guanine nucleotide-binding protein (G protein), alpha-activating activity</td>
</tr>
<tr>
<td><strong>PLCB1</strong></td>
<td>Phospholipase C, beta-1</td>
</tr>
<tr>
<td><strong>SIK1</strong></td>
<td>Salt-inducible kinase 1</td>
</tr>
</tbody>
</table>

**Transcription and gene expression related**

<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PUM1</strong></td>
<td>Pumilio RNA binding family member 1</td>
</tr>
<tr>
<td><strong>TSEN2</strong></td>
<td>tRNA splicing endonuclease, subunit 2</td>
</tr>
<tr>
<td><strong>PURA</strong></td>
<td>Purine-rich element binding protein A</td>
</tr>
<tr>
<td><strong>MEF2C</strong></td>
<td>MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)</td>
</tr>
<tr>
<td><strong>KMT2E</strong></td>
<td>Lysine (K)-specific methyltransferase 2E</td>
</tr>
<tr>
<td><strong>CELF2</strong></td>
<td>CUGbp- and ELAV-like family, member 2</td>
</tr>
<tr>
<td><strong>CUX2</strong></td>
<td>Cut-like homeobox 2</td>
</tr>
<tr>
<td><strong>FOXG1</strong></td>
<td>Forkhead box G1B</td>
</tr>
<tr>
<td><strong>IRF2BPL</strong></td>
<td>Interferon regulatory factor 2-binding protein like</td>
</tr>
<tr>
<td><strong>CHD2</strong></td>
<td>Chromodomain helicase DNA binding protein-2</td>
</tr>
<tr>
<td><strong>NEUROD2</strong></td>
<td>Neurogenic differentiation 2</td>
</tr>
<tr>
<td><strong>MECP2</strong></td>
<td>Methyl-CpG-binding protein-2</td>
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</table>

**Protein biosynthesis/degradation related**

<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
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<tbody>
<tr>
<td><strong>DHDDS</strong></td>
<td>Dehydrodolichyl diphosphate synthase</td>
</tr>
<tr>
<td><strong>ATP6V1A</strong></td>
<td>ATPase, H+ transporting, V1 subunit A</td>
</tr>
<tr>
<td><strong>UBA5</strong></td>
<td>Ubiquitin-like modifier activating enzyme 5</td>
</tr>
<tr>
<td><strong>GUF1</strong></td>
<td>GUF1 homolog, GTPase</td>
</tr>
<tr>
<td><strong>VARS1</strong></td>
<td>Valyl-tRNA synthetase 1</td>
</tr>
<tr>
<td><strong>PLAA</strong></td>
<td>Phospholipase A2-activating protein</td>
</tr>
<tr>
<td><strong>CARS2</strong></td>
<td>Cysteinyl-tRNA synthetase 2</td>
</tr>
<tr>
<td>Approved Symbol</td>
<td>Gene/Locus name</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>AARS1</td>
<td>Alanyl-tRNA synthetase 1</td>
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<tr>
<td>EEF1A2</td>
<td>Eukaryotic translation elongation factor-1, alpha-2</td>
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<tr>
<td>PIGP</td>
<td>Phosphatidylinositol glycan, class P</td>
</tr>
<tr>
<td>PIGA</td>
<td>Phosphatidylinositol glycan, class A</td>
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**Cytoskeletal proteins**

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<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
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<tbody>
<tr>
<td>CYFIP2</td>
<td>Cytoplasmic FMRP interacting protein 2</td>
</tr>
<tr>
<td>PHACTR1</td>
<td>Phosphatase and actin regulator 1</td>
</tr>
<tr>
<td>SPTAN1</td>
<td>Spectrin, alpha, nonerythrocytic-1 (alpha-fodrin)</td>
</tr>
</tbody>
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**Mitochondria proteins**

<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
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<tbody>
<tr>
<td>MFF</td>
<td>Mitochondrial fission factor</td>
</tr>
<tr>
<td>FARS2</td>
<td>Phenylalanyl-tRNA synthetase 2, mitochondrial</td>
</tr>
<tr>
<td>RMND1</td>
<td>Required for meiotic nuclear division 1 homolog</td>
</tr>
<tr>
<td>BRAT1</td>
<td>BRCA1-associated ATM activator 1</td>
</tr>
<tr>
<td>PMPCB</td>
<td>Peptidase, mitochondrial processing, beta</td>
</tr>
<tr>
<td>TWNK</td>
<td>Twinkle mtDNA helicase</td>
</tr>
<tr>
<td>DNM1L</td>
<td>Dynamin 1-like</td>
</tr>
<tr>
<td>POLG</td>
<td>Polymerase (DNA directed), gamma</td>
</tr>
<tr>
<td>GOT2</td>
<td>Glutamic-oxaloacetic transaminase 2, mitochondrial</td>
</tr>
<tr>
<td>TIMM50</td>
<td>Translocase of inner mitochondrial membrane 50</td>
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**Other/multiple functions proteins**

<table>
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<tr>
<th>Approved Symbol</th>
<th>Gene/Locus name</th>
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<tbody>
<tr>
<td>FBXO11</td>
<td>F-box only protein 11</td>
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<tr>
<td>MBD5</td>
<td>Methyl-CpG-binding domain protein 5</td>
</tr>
<tr>
<td>PTPN23</td>
<td>Protein-tyrosine phosphatase, nonreceptor-type, 23</td>
</tr>
<tr>
<td>DALRD3</td>
<td>DALR anticodon-binding domain-containing protein 3</td>
</tr>
<tr>
<td>SERPINI1</td>
<td>Pro tease inhibitor 12</td>
</tr>
<tr>
<td>FGF12</td>
<td>Fibroblast growth factor-12</td>
</tr>
<tr>
<td>CSNK2B</td>
<td>Casein kinase-2, beta polypeptide</td>
</tr>
<tr>
<td>Approved Symbol</td>
<td>Gene/Locus name</td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>CNPY3</strong></td>
<td>Canopy 3, zebrafish, homolog of</td>
</tr>
<tr>
<td><strong>CDK19</strong></td>
<td>Cyclin-dependent kinase 19</td>
</tr>
<tr>
<td><strong>TRRAP</strong></td>
<td>Transformation/transcription domain-associated protein</td>
</tr>
<tr>
<td><strong>FRRS1L</strong></td>
<td>Ferric-chelate reductase 1-like</td>
</tr>
<tr>
<td><strong>COQ4</strong></td>
<td>Coenzyme Q4, S. cerevisiae, homolog of</td>
</tr>
<tr>
<td><strong>ATN1</strong></td>
<td>Atrophin 1</td>
</tr>
<tr>
<td><strong>PACS2</strong></td>
<td>Phosphofurin acidic cluster sorting protein 2</td>
</tr>
<tr>
<td><strong>PIGB</strong></td>
<td>Phosphatidylinositol glycan, class B</td>
</tr>
<tr>
<td><strong>PIGQ</strong></td>
<td>Phosphatidylinositol glycan anchor biosynthesis class Q protein</td>
</tr>
<tr>
<td><strong>ROGDI</strong></td>
<td>Rogdi atypical leucine zipper</td>
</tr>
<tr>
<td><strong>WWOX</strong></td>
<td>WW domain-containing oxidoreductase</td>
</tr>
<tr>
<td><strong>PIGS</strong></td>
<td>Phosphatidylinositol glycan, class S</td>
</tr>
<tr>
<td><strong>TCF4</strong></td>
<td>Transcription factor-4 (immunoglobulin transcription factor-2)</td>
</tr>
<tr>
<td><strong>PNKP</strong></td>
<td>Polynucleotide kinase 3' phosphatase</td>
</tr>
<tr>
<td><strong>CDKL5</strong></td>
<td>Cyclin-dependent kinase-like 5 (serine/threonine protein kinase 9)</td>
</tr>
<tr>
<td><strong>CASK</strong></td>
<td>Calcium/calmodulin-dependent serine protein kinase</td>
</tr>
<tr>
<td><strong>SMC1A</strong></td>
<td>Segregation of mitotic chromosomes 1 (SMC1, yeast, homolog of; DXS423E; SB1.8)</td>
</tr>
<tr>
<td><strong>FGF13</strong></td>
<td>Fibroblast growth factor-13</td>
</tr>
<tr>
<td><strong>ARHGEF9</strong></td>
<td>Rho guanine nucleotide exchange factor 9</td>
</tr>
<tr>
<td><strong>PCDH19</strong></td>
<td>Protocadherin 19</td>
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</tbody>
</table>

Genes gathered from OMIM (https://www.omim.org/) interrogated on October 27, 2021, using the keywords ‘epileptic encephalopathy’ and ‘developmental and epileptic encephalopathy’. See Supplemental Table S1 (https://figshare.com/articles/dataset/Guerrini_et_al_Supplemental_Table_S1_xlsx/17694713) for more details.
## TABLE 3. Selected animal models of DEEs

<table>
<thead>
<tr>
<th>Models</th>
<th>Species</th>
<th>Induction method</th>
<th>Spasms, age of onset</th>
<th>Subsequent epilepsy</th>
<th>Behavioral / neurodevelopmental deficits</th>
<th>Response to ACTH/vigabatrin</th>
<th>Model of:</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Models of epileptic spasms (ES)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1. Acute</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMDA</td>
<td>Rat, mouse C57</td>
<td>NMDA i.p., PN7-18</td>
<td>PN7-18</td>
<td>NR</td>
<td><em>Rat:</em> Learning and coordination deficits; <em>3days – 7days post NMDA (mouse):</em> Increased anxiety, impaired motor coordination and poor memory retention</td>
<td>High dose ACTH1, <em>NR</em> after spasms induction</td>
<td>Spasms</td>
<td>(167, 170, 543, 584–587)</td>
</tr>
<tr>
<td>NMDA variants</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>- Prenatal betamethasone</td>
<td>Rat</td>
<td>Betamethasone i.p. G15;</td>
<td>PN12-15</td>
<td>NR</td>
<td>NR after spasms induction</td>
<td>Low dose ACTH1, <em>39;</em> Spasms, ACTH</td>
<td>(168, 537, 538, 542)</td>
<td></td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
</tr>
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<td>--------------------------------</td>
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<tr>
<td>ne / postnatal</td>
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<td></td>
</tr>
<tr>
<td>NMDA</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Prenatal stress / postnatal</td>
<td>Rat</td>
<td>Forced restraint (FR) (G15 or Forced swim test (FST) (G1-parturition); NMDA i.p., PN15</td>
<td>PN15</td>
<td>NR</td>
<td>NR after spasms induction</td>
<td>FST/NMDA: Responds to ACTH₁-₃₉; FR/NMDA: Responds to repeated low dose ACTH₁-₃₉</td>
<td>Spasms, stress</td>
<td>(167, 169)</td>
</tr>
<tr>
<td>NMDA</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Prenatal MAM / postnatal</td>
<td>Rat</td>
<td>MAM (2 doses, G15); NMDA i.p., PN12-15 (1 or 3 doses)</td>
<td>PN12-15</td>
<td>NR</td>
<td>NR after spasms induction</td>
<td>No effect of low dose ACTH; Responds to vigabatrin</td>
<td>Spasms, dysplasias</td>
<td>(588)</td>
</tr>
<tr>
<td>NMDA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Adrenalecto</td>
<td>Rat</td>
<td>Adrenalectomy (PN10); NMDA i.p.</td>
<td>PN11</td>
<td>NR</td>
<td>NR after spasms induction</td>
<td>High dose ACTH₁-₃₉</td>
<td>Spasms</td>
<td>(170)</td>
</tr>
</tbody>
</table>

References:
- FR/NMDA: Forced restraint/NMDA
- FST/NMDA: Forced swim test/NMDA
- MAM: Maternal Adrenalectomy
<table>
<thead>
<tr>
<th>Models</th>
<th>Species</th>
<th>Induction method</th>
<th>Spasms, age of onset</th>
<th>Subsequent epilepsy</th>
<th>Behavioral / neurodevelopmental deficits</th>
<th>Response to ACTH/vigabatrin</th>
<th>Model of:</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>my / postnatal NMDA</td>
<td></td>
<td>(PN11)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>- Tsc1&lt;sup&gt;gflap&lt;/sup&gt;-/+ mouse, postnatal NMDA</td>
<td>Mouse, Tsc1&lt;sup&gt;lox/lox&lt;/sup&gt;-&lt;sup&gt;−&lt;/sup&gt;/&lt;sup&gt;−&lt;/sup&gt; GFAP-Cre knockout C57Bl/6 and SV129</td>
<td>As in Tsc1&lt;sup&gt;gflap&lt;/sup&gt;-/+</td>
<td>NR after spasms induction</td>
<td>NR</td>
<td>Spasms, induced on a genetic background</td>
<td>(589)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down syndrome / GBL</td>
<td>Mouse (Ts65Dn), C57Bl/6JEIXC3H/HesnJ</td>
<td>γ-butyrolactone (GBL) i.p.</td>
<td>1wk – 2mo</td>
<td>NR</td>
<td>NR after spasms induction</td>
<td>Responds to ACTH&lt;sub&gt;1-24&lt;/sub&gt; but not to ACTH&lt;sub&gt;1-39&lt;/sub&gt;; Responds to vigabatrin</td>
<td>Spasms, induced on a genetic background</td>
<td>(539, 590, 591)</td>
</tr>
<tr>
<td>A2. Chronic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tetrodotoxin (TTX)</td>
<td>Rat</td>
<td>Intrahippocampal or intracortical TTX, unilateral</td>
<td>≥PN21</td>
<td>Yes</td>
<td>NR</td>
<td>Sensitive to ACTH, vigabatrin</td>
<td>ISS structural, hypsarrhythm</td>
<td>(87, 546, 592–594)</td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
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<tr>
<td>Multiple-hit</td>
<td>Rat</td>
<td>PN3: Right intracortical LPS, right intraventricular doxorubicin, PNS: PCPA i.p.</td>
<td>PN4-13</td>
<td>Yes</td>
<td>Sociability deficits, learning/memory deficits, stereotypies</td>
<td>Resistant to ACTH; partial/transient response to vigabatrin</td>
<td>ISS, structural, drug-resistant</td>
<td>(88, 155–161)</td>
</tr>
<tr>
<td>Arx cKO</td>
<td>Mouse, CD1 and C57BL/6</td>
<td>Arx deletion from ganglionic eminence neuronal progenitors</td>
<td>Adulthood</td>
<td>Yes</td>
<td>NR</td>
<td>NR</td>
<td>ISS, genetic</td>
<td>(162)</td>
</tr>
<tr>
<td>Arx KI [Arx (GCG)10+7]</td>
<td>Mouse, 75% C57BL/6; 25%</td>
<td>Expansion of 1st polyalanine</td>
<td>PN7-11</td>
<td>Yes</td>
<td>Low anxiety, impaired learning</td>
<td>NR</td>
<td>ISS, genetic</td>
<td>(150, 163, 595)</td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
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<tr>
<td></td>
<td>129S5/SvEvBrd</td>
<td>tract repeat (PA1) of Arx</td>
<td></td>
<td></td>
<td>and sociability</td>
<td></td>
<td></td>
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<tr>
<td>Arx with PA1 or PA2 expansion</td>
<td>Mouse, C57BL/6N-Hsd</td>
<td>PA1 or PA2 expansion of Arx</td>
<td>≥PN10 to adulthood</td>
<td>NR</td>
<td>1-2mo: Sociability, neuromuscular strength deficits, anxiety and fear</td>
<td>NR</td>
<td>ISS, genetic</td>
<td>(545)</td>
</tr>
<tr>
<td>Adenomatous polyposis (Apc)</td>
<td>Mouse</td>
<td>Apc gene knockout from excitatory CamKII neurons</td>
<td>Peak at PN9</td>
<td>Yes</td>
<td>Reduced social interest, increased repetitive behaviors</td>
<td>NR</td>
<td>ISS, genetic</td>
<td>(596)</td>
</tr>
<tr>
<td>Tsc1+/−</td>
<td>Mouse, C57BL/6</td>
<td>Heterozygous Tsc1+/−</td>
<td>PN12-16; Observed for 1 day/pup</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>ISS, genetic</td>
<td>(597)</td>
</tr>
<tr>
<td>Aged CDKL5, heterozygous females</td>
<td>Mouse, C57BL/6J</td>
<td>Cdkl5&lt;sup&gt;lox/lox&lt;/sup&gt; or Cdkl5&lt;sup&gt;ESO/+&lt;/sup&gt;</td>
<td>&gt;PN300 female</td>
<td>No (only spasms seen)</td>
<td>NR in females; Sociability deficits in males</td>
<td>NR</td>
<td>ISS, genetic</td>
<td>(598)</td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
</tr>
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</tr>
<tr>
<td>Chronic early life stress</td>
<td>Rat</td>
<td>Unpredictable and fragmented nurturing behaviors in dams (PN2-9 period)</td>
<td>PN17-35; Last for 1 or several days</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>ISS, unknown</td>
<td>(171)</td>
</tr>
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</tr>
<tr>
<td>B. Models of Lennox-Gastaut syndrome, atypical absence seizures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AY9944</td>
<td>Rat</td>
<td>AY9944 7.5mg/kg SC (PN2, 8, 14, PN20)</td>
<td>NR</td>
<td>Slow SWD</td>
<td>Cognitive deficits, hyperactivity, anxiety, spatial learning, olfactory recognition deficits</td>
<td>Responsive to DZP, ETH, CGP35348; Worse with CZP, baclofen, γ-OH-butyrate</td>
<td></td>
<td>(599–605)</td>
</tr>
<tr>
<td>MAM - AY9944</td>
<td>Rat</td>
<td>Prenatal MAM / postnatal AY9944</td>
<td>NR</td>
<td>Slow SWD</td>
<td>NR</td>
<td>Refractory to ETH, VPA, CGP35348, CBZ</td>
<td>Refractory atypical absence seizures</td>
<td>(606)</td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
</tr>
<tr>
<td>-----------------</td>
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<tr>
<td>PVAlb-</td>
<td>Mouse, C57BL/6J</td>
<td>Dnm1*&lt;sup&gt;fl/fl&lt;/sup&gt; in PV cells</td>
<td>NR</td>
<td>PN19-50: SWD, lethal seizures</td>
<td>Tremor</td>
<td>NR</td>
<td>LGS</td>
<td>(607)</td>
</tr>
<tr>
<td>Dnm1*&lt;sup&gt;fl/fl&lt;/sup&gt;</td>
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<tr>
<td>GABABR1a</td>
<td>Mouse, C57BL/6</td>
<td>GABABR1a overexpression in forebrain</td>
<td>NR</td>
<td>Slow SWD</td>
<td>Impairment in learning, spatial memory</td>
<td>Responsive to ETH, CGP35348</td>
<td>Chronic atypical absence seizures</td>
<td>(608)</td>
</tr>
<tr>
<td>GABABR1b</td>
<td>Mouse</td>
<td>GABABR1b overexpression in forebrain</td>
<td>NR</td>
<td>Slow SWD</td>
<td>Mild impairment in learning, spatial memory</td>
<td>Responsive to ETH, CGP35348</td>
<td>Chronic atypical absence seizures</td>
<td>(609)</td>
</tr>
<tr>
<td>NHE1</td>
<td>Mouse, SJL/J and C57BL/6J</td>
<td>Na+/H+ exchanger null</td>
<td>NR</td>
<td>Slow SWD (3Hz) (at 4-5wk); Lethal tonic or tonic-clonic</td>
<td>Ataxia; Early mortality</td>
<td>NR</td>
<td>Chronic atypical absence seizures</td>
<td>(610)</td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
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<tr>
<td>Multiple-hit</td>
<td>Rat</td>
<td>PN3: Right intracortical LPS, right intraventricular doxorubicin PN5: PCPA i.p.</td>
<td>PN4-13</td>
<td>Adulthood: slow SWD (5-6Hz), motor seizures in sleep</td>
<td>Sociability deficits, learning/memory deficits, stereotypes</td>
<td>NR</td>
<td>ISS with LGS features</td>
<td>(155)</td>
</tr>
<tr>
<td>C. Models of Dravet syndrome</td>
<td>Mouse</td>
<td>Exon 26 deletion, global constitutive</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Scn1a KO</td>
<td>Mouse, C57BL/6 and</td>
<td>Exon 25 deletion,</td>
<td>Convulsive seizures, Hyperthermia seizures, mortality</td>
<td>Hyperactivity, stereotypies, sociability, and spatial memory deficits</td>
<td>Tested</td>
<td>Dravet</td>
<td>(64)</td>
<td></td>
</tr>
<tr>
<td>Scn1a CKO</td>
<td></td>
<td>Exon 25 deletion,</td>
<td>Motor</td>
<td>NR</td>
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<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
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<tr>
<td>129Sv</td>
<td>forebrain GABAergic interneurons</td>
<td>seizures, Hyperthermia seizures</td>
<td></td>
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<tr>
<td><strong>Scn1a CKO</strong></td>
<td>Mouse, B6.SJL-Tg(ACTFLPe)9205Dym/J and C57BL/6</td>
<td>Conditional deletion of exon 7 Inhibitory neurons</td>
<td>≥PN16 seizures, occasional death</td>
<td>Hypoactive, jerks, death</td>
<td>Dravet</td>
<td>(612)</td>
<td></td>
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<tr>
<td></td>
<td>Forebrain excitatory neurons</td>
<td>No</td>
<td>NR</td>
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<td></td>
<td>Forebrain excitatory neurons and haploinsufficiency in inhibitory</td>
<td>Improved lethality from seizures</td>
<td>NR</td>
<td>Dravet</td>
<td>(612)</td>
<td></td>
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<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
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<td></td>
<td></td>
<td>PV interneurons</td>
<td>≥PN14 seizures, death</td>
<td></td>
<td>Ataxia (PN10)</td>
<td></td>
<td>Dravet</td>
<td>(612)</td>
</tr>
<tr>
<td><em>Scn1a KI, R1407X</em></td>
<td>Mouse, 129/SvJ and CS7BL/6J</td>
<td>Human R1407X nonsense mutation</td>
<td>≥1mo: Seizures</td>
<td></td>
<td>Hyperactivity, stereotypies, sociability and</td>
<td></td>
<td>Dravet</td>
<td>(65, 224, 225)</td>
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<tr>
<td><em>Scn1a KI, S1231R</em></td>
<td>Drosophila</td>
<td>S1231R mutation, loss of function</td>
<td>Seizures</td>
<td>NR</td>
<td></td>
<td></td>
<td>Dravet</td>
<td>(613)</td>
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<tr>
<td><em>Scn1Lab (didy&lt;sup&gt;ss2&lt;/sup&gt;)</em></td>
<td>Zebrafish</td>
<td>Scn1Lab mutation (low expression)</td>
<td>Increased locomotor activity, epileptiform activity,</td>
<td>Impaired exploration, decreased mobility</td>
<td>Tested</td>
<td></td>
<td>Dravet</td>
<td>(614)</td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
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</tr>
</tbody>
</table>
| Scn1Lab
t-/- | Zebrafish | Scn1Lab null | | | Increased locomotor activity and epileptiform activity | NR | Tested | Dravet | (560) |
| Scn1a- A1783V/WT KI | Mouse, C57BL/6J | Scn1a- A1783V/WT KI | | | Hyperthermia seizures | NR | Tested | Dravet | (615) |
| Scn1a R1648H KI (after induction of short seizures) | Mouse | Knock-in R1658H missense mutation, global constitutive, | Convulsive seizures, Hyperthermia seizures, mortality | Hyperactivity, stereotypies, sociability, and spatial memory deficits | Tested | Dravet and GEFS+ | (228) |

D. Other etiology:
<table>
<thead>
<tr>
<th>Models</th>
<th>Species</th>
<th>Induction method</th>
<th>Spasms, age of onset</th>
<th>Subsequent epilepsy</th>
<th>Behavioral / neurodevelopmental deficits</th>
<th>Response to ACTH/vigabatrin</th>
<th>Model of:</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>specific models of DEE</td>
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<tr>
<td><em>Kcnq2</em> KI</td>
<td>Mouse, C57BL/6J</td>
<td>Kcnq2-Y284C/+, Kcnq2-A306T/+</td>
<td>NR</td>
<td>NR</td>
<td>Retigabine reduces KA-seizures</td>
<td>KCNQ2 DEE</td>
<td>(616)</td>
<td></td>
</tr>
<tr>
<td><em>Kcnq2-Thr274Met/+</em></td>
<td>Mouse, 129Sv, C57BL/6N</td>
<td>Kcnq2-Thr274Met/+</td>
<td>Yes (&gt;PN20)</td>
<td>Spatial learning and memory deficits. Death by 3rd mo (25%)</td>
<td>NR</td>
<td>KCNQ2 DEE</td>
<td>(281)</td>
<td></td>
</tr>
<tr>
<td><em>Kcna1/-</em></td>
<td>Mouse</td>
<td><em>Kcna1/-</em></td>
<td>Yes</td>
<td>NR</td>
<td>Retigabine reduces KCNQ1 epilepsy</td>
<td></td>
<td>(617)</td>
<td></td>
</tr>
<tr>
<td><em>Kcnq1</em></td>
<td>Mouse</td>
<td><em>Kcnq1</em></td>
<td>Rare</td>
<td>NR</td>
<td>Retigabine:</td>
<td>KCNQ1</td>
<td>(617)</td>
<td></td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
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<tr>
<td>A340E/A340E</td>
<td>A340E/A340E</td>
<td>spontaneous seizures</td>
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<td>adverse cardiac effects</td>
<td>epilepsy</td>
<td></td>
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<tr>
<td><strong>Pcdh19 KO and heterozygous females</strong></td>
<td>Mouse, 129S5.C57BL/6</td>
<td><strong>Pcdh19 KO and heterozygous females</strong></td>
<td></td>
<td>NR; Increased susceptibility to 6Hz and flurothyl seizures</td>
<td>Pattern completion and separation deficits</td>
<td>NR</td>
<td><strong>PCDH19 DEE</strong></td>
<td>(618)</td>
</tr>
<tr>
<td><strong>Pcdh19-HET</strong></td>
<td>Mouse, C57BL/6N</td>
<td><strong>Pcdh19-HET</strong></td>
<td>Mossy fiber deficits</td>
<td>NR</td>
<td>Increased exploratory behavior, reduced anxiety</td>
<td>NR</td>
<td><strong>PCDH19 DEE</strong></td>
<td>(619)</td>
</tr>
<tr>
<td><strong>Pcdh19 KO</strong></td>
<td>Mouse, C57BL/6N</td>
<td><strong>Pcdh19-KO</strong></td>
<td>NR</td>
<td>NR</td>
<td>Increased exploratory behavior, reduced anxiety</td>
<td>NR</td>
<td><strong>PCDH19 DEE</strong></td>
<td>(618)</td>
</tr>
<tr>
<td>Models</td>
<td>Species</td>
<td>Induction method</td>
<td>Spasms, age of onset</td>
<td>Subsequent epilepsy</td>
<td>Behavioral / neurodevelopmental deficits</td>
<td>Response to ACTH/vigabatrin</td>
<td>Model of:</td>
<td>References</td>
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<tr>
<td>Cdkl5 CKO</td>
<td>Mouse, CD1</td>
<td>Cdkl5 CKO in glutamatergic or GABAergic neurons</td>
<td>Defective dendritic arborisation and spine maturation</td>
<td>Yes when deleted in glutamatergic neurons.</td>
<td>Autistic symptomatology, motor coordination, memory and breathing abnormalities</td>
<td>Epigallatocatechin-3-gallate (EGCG) corrects synaptic deficits</td>
<td>CDKL5 DEE</td>
<td>(620, 621)</td>
</tr>
</tbody>
</table>
aspartate; NR: not reported; PA: polyalanine; PCPA: p-chlorophenylalanine (inhibits serotonin synthesis); PN: postnatal; PV or PValb: parvalbumin;

Scn1a: sodium channel 1 alpha; SWD: spike and slow wave discharge; Tsc: tuberous sclerosis complex; TTX: tetrodotoxin; VPA: valproic acid.
### TABLE 4. Experimental drugs tested in models of DEE

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
<th>Model / species</th>
<th>Treatment Protocol</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. ES models</strong></td>
<td></td>
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<tr>
<td>CGP35348</td>
<td>GABA_(\beta)R antagonism</td>
<td>Mouse, Down/GBL</td>
<td>Pre-treatment</td>
<td>Shortens EDRs</td>
<td>(538)</td>
</tr>
<tr>
<td>Baclofen</td>
<td>GABA_(\beta)R agonist</td>
<td>Mouse, Down/GBL</td>
<td>Pre-treatment</td>
<td>Prolongs EDRs</td>
<td>(538)</td>
</tr>
<tr>
<td>5-OH-tryptophan</td>
<td>Serotonin increase</td>
<td>Mouse, Down/GBL</td>
<td>Pre-treatment</td>
<td>Prolongs EDRs</td>
<td>(538)</td>
</tr>
<tr>
<td>GIRK2 knockout</td>
<td>Deletion of GABA_(\beta)R</td>
<td>GIRK2 KO Mouse, Down/GBL</td>
<td>GIRK2 Knockout mice</td>
<td>GIRK2 knockout confers resistance to GBL induced spasms</td>
<td>(539)</td>
</tr>
<tr>
<td></td>
<td>associated inward rectifying</td>
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<tr>
<td></td>
<td>potassium channel</td>
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<tr>
<td>Rapamycin</td>
<td>mTOR inhibitor</td>
<td>Rat, Prenatal betamethasone / postnatal NMDA</td>
<td>Pre-treatment</td>
<td>No effect, no evidence of target relevance</td>
<td>(537)</td>
</tr>
<tr>
<td>Ganaxolone</td>
<td>Allosteric</td>
<td>Rat, Prenatal</td>
<td>Pre-treatment</td>
<td>Reduces number,</td>
<td>(540)</td>
</tr>
<tr>
<td>Drug</td>
<td>Mechanism</td>
<td>Model / species</td>
<td>Treatment Protocol</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>ACTON PROLONGATUM *</td>
<td>activator of GABA_{2}R, synaptic and extrasynaptic</td>
<td>betamethasone / postnatal NMDA</td>
<td></td>
<td>delays onset of NMDA spasms</td>
<td>(541)</td>
</tr>
<tr>
<td>AQB-565</td>
<td>Synthetic ACTH based on porcine ACTH</td>
<td>Rat, Prenatal betamethasone / postnatal NMDA</td>
<td>Pre-treatment</td>
<td>Reduces number of NMDA spasms after 8 doses</td>
<td>(538)</td>
</tr>
<tr>
<td>Estradiol, diethylstilbestrol</td>
<td>ACTH1-24 linked to melanocyte stimulating hormone, acts on MC3, MC4 melanocortin receptors</td>
<td>Rat, Prenatal betamethasone / postnatal NMDA</td>
<td>Pre-treatment</td>
<td>Reduces number of NMDA spasms after 10 doses</td>
<td>(542)</td>
</tr>
<tr>
<td>Drug</td>
<td>Mechanism</td>
<td>Model / species</td>
<td>Treatment Protocol</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>estrogen analogue</td>
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<td>postnatal NMDA</td>
<td></td>
<td>GAD67 cells in neocortex</td>
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</tr>
<tr>
<td><strong>β-OH-butyrate</strong></td>
<td>Ketoacid</td>
<td>Rat, Prenatal betamethasone / postnatal NMDA</td>
<td>Pre-treatment</td>
<td>Reduces spasms and delays latency to NMDA spasms after repeat but not single dose administration</td>
<td>(543)</td>
</tr>
<tr>
<td><strong>β-OH-butyrate</strong></td>
<td>Ketoacid</td>
<td>Rat, Prenatal betamethasone / postnatal NMDA</td>
<td>Pre-treatment (200mg/kg ip)</td>
<td>No effect</td>
<td>(544)</td>
</tr>
<tr>
<td>2-deoxyglucose</td>
<td>Metabolic inhibition of glycolysis</td>
<td>Rat, Prenatal betamethasone / postnatal NMDA</td>
<td>Pre-treatment</td>
<td>No effect</td>
<td>(544)</td>
</tr>
<tr>
<td><strong>B. ISS models</strong></td>
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<tr>
<td><strong>Rapamycin, pulse</strong></td>
<td>mTOR inhibitor</td>
<td>Rat, multiple hit</td>
<td>Treatment after spasms onset, 3days</td>
<td>Decreases spasms within 2h; stops</td>
<td>(155, 161)</td>
</tr>
<tr>
<td>Drug</td>
<td>Mechanism</td>
<td>Model / species</td>
<td>Treatment Protocol</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>CPP-115</td>
<td>High affinity vigabatrin analog</td>
<td>Rat, multiple hit</td>
<td>Treatment after spasms onset, repeated (PN4-12)</td>
<td>Reduces spasms from the first hour and for up to 3 days; better efficacy and tolerability than vigabatrin</td>
<td>(156)</td>
</tr>
<tr>
<td>Carisbamate</td>
<td>Unknown; effect on spasms not due to sodium channel blockade</td>
<td>Rat, multiple hit</td>
<td>Treatment after spasms onset, single dose (PN4 or PN6-7)</td>
<td>Reduces spasms within the first hour</td>
<td>(160)</td>
</tr>
<tr>
<td>Drug</td>
<td>Mechanism</td>
<td>Model / species</td>
<td>Treatment Protocol</td>
<td>Effect</td>
<td>Reference</td>
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<tr>
<td>NAX-5055</td>
<td>Galanin receptor 1 (GalR1) agonist</td>
<td>Rat, multiple hit</td>
<td>Treatment after spasms onset, single dose (PN4 or PN6-7)</td>
<td>No effect; low expression of GalR1 in pups</td>
<td>(158)</td>
</tr>
<tr>
<td>VX-765</td>
<td>Caspase 1 inhibitor</td>
<td>Rat, multiple hit</td>
<td>Treatment after spasms onset, single dose (PN4)</td>
<td>No effect</td>
<td>(157)</td>
</tr>
<tr>
<td>CGP 35348</td>
<td>GABA&lt;sub&gt;9&lt;/sub&gt;R antagonism</td>
<td>Rat, multiple hit</td>
<td>Treatment after spasms onset, single dose (PN4)</td>
<td>No effect</td>
<td>(157)</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>Gonadal hormone</td>
<td>Rat, multiple hit</td>
<td>Treatment started after induction (PN3-10)</td>
<td>No effect</td>
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<td>17β-estradiol</td>
<td>Gonadal hormone</td>
<td>Mouse, Arx KI [Arx (GCG&lt;sup&gt;10/7&lt;/sup&gt;)</td>
<td>Pre-treatment (PN3-10)</td>
<td>Prevents spasms and other</td>
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<tr>
<td>17β-estradiol</td>
<td>Gonadal hormone</td>
<td>Mouse, Arx with PA1 or PA2 expansion</td>
<td>Pre-treatment (PN3-10)</td>
<td>Reduces seizures but not the</td>
<td>(545)</td>
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<td>Soticlestat</td>
<td>Cholesterol 24-hydroxylase inhibitor</td>
<td>Mouse, Scn1a&lt;sup&gt;tm1Kea&lt;/sup&gt; with exon 1 deletion</td>
<td>Treatment after hyperthermia priming</td>
<td>Reduced seizures, protected against hyperthermia seizures, prevented SUDEP</td>
<td>(549)</td>
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<tr>
<td>Medium chain triglyceride diet (decanoic C10, octanoic acid C8 mix)</td>
<td>Ketogenic diet metabolite</td>
<td>Mouse, Scn1a&lt;sup&gt;KI&lt;/sup&gt;, R1407X</td>
<td>4wk treatment prior to hyperthermia</td>
<td>C10/C8 (80:20) reduce both seizures and mortality; C10 reduces mortality</td>
<td>(551)</td>
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<td>Gabra2 repair</td>
<td>GABRA2 expression restoration (increase)</td>
<td>Mouse, Scn1a&lt;sup&gt;/−/−&lt;/sup&gt;</td>
<td>Genetic repair of Gabra2</td>
<td>Rescues epilepsy phenotype</td>
<td>(550)</td>
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C. Dravet syndrome models
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<td><em>Trpv1 receptor deletion</em></td>
<td><em>Trpv1 receptor deletion</em></td>
<td><em>Mouse, Scn1a&lt;sup&gt;+/−&lt;/sup&gt;</em></td>
<td><em>Trpv1 receptor deletion</em></td>
<td>No effects on hyperthermia seizures, frequency of spontaneous seizures or survival</td>
<td>(552)</td>
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<td><strong>SB-70549</strong></td>
<td><em>Trpv1 selective antagonist</em></td>
<td><em>Mouse, Scn1a&lt;sup&gt;+/−&lt;/sup&gt;</em></td>
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<td>No effect on seizures or survival</td>
<td>(552)</td>
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<td><strong>Cannabigerolic acid</strong></td>
<td><em>Phytocannabinoid</em></td>
<td><em>Mouse, Scn1a&lt;sup&gt;+/−&lt;/sup&gt;</em></td>
<td><em>Pre-treatment</em></td>
<td>Potentiated clobazam effects on hyperthermia induced and spontaneous seizures, anticonvulsant in MES, proconvulsant in 6Hz test.</td>
<td>(554)</td>
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<td>Cannabichromene, 5-Fluoro- Cannabichromene</td>
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<td>Pre-treatment</td>
<td>Anticonvulsant</td>
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<td>Ketogenic diet</td>
<td>Ketogenic diet</td>
<td>*Mouse, Scn1α KI, R1407X</td>
<td>Ketogenic diet, 14 days</td>
<td>Decreases SUDEP, protects against seizure induced respiratory arrest</td>
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<td>SCN1A transfer in the brain (adenoviral)</td>
<td>SCN1A expression in the brain</td>
<td><em>Mouse, SCN1A-A1783V KI</em></td>
<td>Adenovirus expressing SCN1A, intracerebral injection</td>
<td>Protected from death, attenuation of epilepsy, hyperactivity persisted, cognitive effects variable</td>
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<td>Naltrexone</td>
<td>Opioid antagonist</td>
<td><em>Zebrafish, scn1Lab</em></td>
<td>Pretreatment, 30min prior to PTZ</td>
<td>Anticonvulsant effects</td>
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<td>Fenfluramine, Norflufenfluramine</td>
<td>Reuptake inhibitor of 5-OH-</td>
<td><em>Zebrafish, scn1Lab-/-</em></td>
<td>Treatment exposure</td>
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<td>Dimethadione</td>
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<td>Pck1 activator and translocator protein ligand</td>
<td>Zebrafish, <em>scn1Lab</em></td>
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<td>GR-46611</td>
<td>5HT1D receptor agonist</td>
<td>Mouse, 129S-Scn1Atm1Kea/Mmjax</td>
<td>Pretreatment</td>
<td>Protects from hyperthermia, decreases seizure severity, improves survival</td>
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<td>ASO, increases</td>
<td>Mouse, F1:129S-PN2 or 14,</td>
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<td>Scn1a</td>
<td>Scn1a (TANGO method)</td>
<td>Scn1a+/− Å~ C57BL/6J</td>
<td>intracerebroventricular injection</td>
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<td>CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons</td>
<td>CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons</td>
<td>Mouse, Scn1aΔKO, floxed dCas9-VPRVPR+/+, Vgat-CreCre+/+.</td>
<td>Intravenous AAV injection, 4wk</td>
<td>Improved behavioral deficits, ameliorated febrile seizures</td>
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<td>MV1369, MV1312</td>
<td>NaV1.6 inhibitor</td>
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<td>AA43279</td>
<td>Nav1.1 activator</td>
<td>Zebrafish, Scn1Lab KO</td>
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3794 AQB-565: ACTH1-24 linked to melanocyte stimulating hormone; ASO: antisense oligonucleotides; CPP-115: high affinity GABA aminotransferase inhibitor; CRISPR: clustered regularly interspaced short palindromic repeats; EDRs: electrodecremental responses; GABRA2: GABA\textsubscript{A}R A2 subunit; 3795 CGP35348: GABA\textsubscript{B} receptor inhibitor; DEE: Developmental and epileptic encephalopathy; ES: epileptic spasms; GABA\textsubscript{B}R: GABA\textsubscript{B} receptor; GalR:
galanin receptor; GBL: gamma butyrolactone; GIRK: inward rectifying potassium channel; 5-HT: serotonin; Kl: knockin; mTOR: mechanistic target of rapamycin; MC: melanocortin receptor; Nav: Sodium channel; NMDA: N-methyl-D-aspartate; PCK1: Phosphoenolpyruvate Carboxykinase 1; PN: postnatal; Scn1a sodium channel 1; SUDEP: sudden death in epilepsy; Trpv1: transient receptor potential cation channel subfamily V member 1; Vgat: vesicular GABA transporter.
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<td></td>
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<tr>
<td>Zonisamide</td>
<td>YES</td>
<td>YES</td>
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<td></td>
<td></td>
<td></td>
<td>YES</td>
<td>FS, GTCs, MYO</td>
<td></td>
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</table>

BS: broad spectrum; GABA: Gamma-aminobutyric acid; 5-TH: 5-hydroxytryptamine; SV2A: synaptic vesicle protein 2°; FS: focal seizures; LGS: Lennox Gastaut syndrome; DS: Dravet syndrome; GTCS: generalized tonic-clonic seizures; ABS: absence; SGS: secondary generalized seizures; ES: epileptic spasms; MYO: myoclonic.

### TABLE 6. Clinical trials on DEEs.

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<th>Study Title: Study to Evaluate NBI-921352 as Adjunctive Therapy in Subjects With SCN8A Developmental and Epileptic Encephalopathy Syndrome (SCN8A-DEE)</th>
<th>Recruiting: Not yet</th>
<th>Has results: No</th>
<th>Conditions: SCN8A DEE Syndrome</th>
<th>Interventions: Drug: NBI-921352, Placebo</th>
<th>Locations: Neurocrine Clinical Site, Washington, District of Columbia, United States</th>
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<td></td>
<td>Study Title</td>
<td>Recruiting</td>
<td>Has results</td>
<td>Conditions</td>
<td>Interventions</td>
<td>Locations</td>
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<td>3</td>
<td><strong>Study Title:</strong> An Open-Label Extension of the Study XEN496 (Ezogabine) in Children With KCNQ2-DEE</td>
<td>Yes</td>
<td>No</td>
<td>Epilepsy, Epilepsy in Children, Seizure Disease, Brain Diseases, Central Nervous System Diseases, Nervous System Diseases, Epileptic Syndromes</td>
<td>Drug: XEN496, Placebo</td>
<td>MultiCare Health System - Mary Bridge Pediatrics - Tacoma, Tacoma, Washington, United States.</td>
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<td>4</td>
<td><strong>Study Title:</strong> Study of TAK-935 as an Adjunctive Therapy in Participants With Developmental and/or Epileptic Encephalopathies</td>
<td>No</td>
<td>Yes</td>
<td>Developmental and/or Epileptic Encephalopathies</td>
<td>Drug: TAK-935, Placebo</td>
<td>Xenoscience, Phoenix, Arizona, United States. Medsol Clinical Research Center, Port Charlotte, Florida, United States. University of South Florida, Tampa, Florida, United States. Center for Integrative Rare Disease Research, Atlanta, Georgia, United States. Bluegrass Epilepsy Research, Lexington, Kentucky, United States. Mid-Atlantic Epilepsy and Sleep Center, Bethesda, Maryland, United States. The Comprehensive Epilepsy Care Center for Children and Adults, Saint Louis, Missouri, United States. Northeast Regional Epilepsy Group, Hackensack, New Jersey, United States. Thomas Jefferson University, Philadelphia, Pennsylvania, United States. Medical University of South Carolina, Charleston, South Carolina, United States. University of Virginia Health Sciences Center, Charlottesville, Virginia, United States.</td>
</tr>
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<td>5</td>
<td><strong>Study Title:</strong> A Phase 2, Multicenter, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy, Safety, and Tolerability of TAK-935 (OV935) as an Adjunctive Therapy In Pediatric Participants With Developmental and/or Epileptic Encephalopathies</td>
<td>No</td>
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Has results: Yes

Conditions: Epilepsy, Dravet Syndrome, Lennox-Gastaut Syndrome

Interventions: Drug: TAK-935, Placebo

Locations: Phoenix Children's Hospital, Phoenix, Arizona, United States. Children's Hospital Los Angeles, Los Angeles, California, United States. Colorado Children's Hospital, Aurora, Colorado, United States. Nicklaus Children's Hospital, Miami, Florida, United States. Pediatric Neurology PA, Orlando, Florida, United States. Rare Disease Research, LLC, Atlanta, Georgia, United States. Center for Rare Neurological Diseases, Norcross, Georgia, United States. Ann and Robert H Lurie Childrens Hospital of Chicago, Chicago, Illinois, United States. Mayo Clinic - PPDS, Rochester, Minnesota, United States. Northeast Regional Epilepsy Group, Hackensack, New Jersey, United States. Children's Hospital at Saint Peter's University Hospital, New Brunswick, New Jersey, United States. Columbia University Medical Center, New York, New York, United States. Wake Forest Baptist Medical Center, Winston-Salem, North Carolina, United States. Medical University of South Carolina, Charleston, South Carolina, United States. Cook Children's Medical Center, Fort Worth, Texas, United States. Monash Children's Hospital, Clayton, Victoria, Australia. Austin Hospital, Heidelberg West, Victoria, Australia. Hospital For Sick Children, Toronto, Ontario, Canada. Peking University First Hospital, Beijing, China. Capital Medical University (CMU) - Beijing Children's Hospital, Beijing, China. Beijing Children's Hospital, Capital Medical University, Beijing, China. Xiangya Hospital Central South University, Changsha, China. Children's Hospital of Fudan University, Shanghai, China. Shenzhen Children's Hospital, Shenzhen, China. Sheba Medical Center-PPDS, Tel Hashomer,, Ramat Gan, Israel. Soroka University Medical Centre, Beer Sheva, Israel. Bnai Zion Medical Center, Haifa, Israel. Edith Wolfson Medical Center, Holon, Israel. Hadassah Medical Center, Jerusalem, Israel. Schneider Childrens Medical Center of Israel, Petach Tikva, Israel. Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. Uniwersyteckie Centrum Kliniczne - PPDS, Gdansk, Pomorskie, Poland. NZOZ Centrum Neurologii Dzieciej i Leczenia Padaczkii, Kielce, Swietokrzyskie, Poland. Szpital Kliniczny im. H. Swiecickiego Uniwersytetu Medycznego im. Karola Marcinkowskiego w Poznaniu, Poznan, Wielkopolskie, Poland. Centrum Medyczne Plejady, Krakow, Poland. Samodzielny Publiczny Dzieciecy Szpital Kliniczny w Warszawie, Warsaw, Poland. Instytut Pomnik Centrum Zdrowia Dziecka, Warsaw, Poland. Centro Hospitalar Lisboa Central- Hospital Dona Estefania, Lisboa, Portugal. Centro Hospitalar Lisboa Norte, E.P.E. Hospital de Santa Maria, Lisboa, Portugal. Largo da
Clinical Trials gathered from ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home) interrogated on October 27, 2021 using the keywords ‘developmental and epileptic encephalopathy’.
**Figures Legends**

**FIGURE 1. Schematic representation of an example of ‘poison exon’-mediated protein degradation.** Panel A shows a hypothetical gene encoding a transmembrane protein with four transmembrane segments. The gene comprises nine coding exons (1-9) and a potential poison exon (P). In the canonical splicing, the poison exon is not included in the mRNA, which is translated into the wild type protein. After translation, the protein is correctly integrated into the plasma membrane, where it exerts its normal function. In panel B, the presence of an intronic mutation, which can introduce a novel splicing acceptor site, activate an exonic splicing enhancer (ESE, i.e. a sequence that promotes the inclusion of an exon in an mRNA) or disrupt an exonic splicing silencer (ESS, i.e. a sequence that inhibits the inclusion of an exon in an mRNA), promoting the inclusion of the poison exon in the mRNA. The poison exon alters protein amino acid sequence and introduces a premature stop codon (PTC). The PTC is recognized by cellular surveillance systems and the mutant protein is degraded.

**FIGURE 2. Schematic representation of the cortical and subcortical zones and mechanisms involved in epileptic seizures generation and spreading.** A. Schematic model of networks involved in spike-and-wave generation in generalized epilepsies. Thalamic relay neurons in the thalamocortical circuit can activate cortical pyramidal neurons and *vice versa*. Thalamus-mediated cortical activation is largely controlled by thalamic reticular neurons. They hyperpolarize thalamic relay neurons through gamma-aminobutyric acid type B (GABA$_B$)-mediated signals and are themselves inhibited by neighboring reticular neurons through GABA type A (GABA$_A$)-mediated signals. Cortical pyramidal neurons can, in turn, activate thalamic reticular neurons in a glutamate-mediated feed-forward loop. The neuronal basis of the EEG spike-and-wave in this reverberating loop derive from an alternance of the summated outside-negative excitatory
membrane events (each spike) and the summated outside-positive inhibitory membrane events (each slow wave). Spike and waves appear as negative (upward going) events due to a dipole effect as the soma and apical dendrites maintain opposite polarity. B. In the epileptic brain, focal epileptic seizures are generated in the epileptogenic zone (EZ), while clinical seizures are generated in the seizure onset zone (SOZ). If the EZ is larger than the SOZ, as in the case depicted in the figure, its complete removal is required to guarantee seizures disappearing as multiple SOZs with different thresholds may coexist in the same EZ. The complete EZ disconnection or removal is also required to ensure that seizures do not spread to other areas connected to it via cortico-cortical and subcortical (i.e. thalamocortical) connections (purple arrows), which can cause secondary generalization. Additional specific cortical areas that can be identified in the epileptic brain are the epileptogenic lesion (EL), which may correspond to either a macroscopic epileptogenic lesion (e.g. focal cortical dysplasia, as shown in the figure) or hyperexcitable adjacent cortex, the irritative zone (IZ), representing the area of the normal cortex generating interictal spikes, and the functional deficit zone (FDZ), representing the area of the cortex that does not function normally in the interictal period.

**FIGURE 3. Trajectories of developmental processes in normal brain development.** A. The temporal trajectories of selected developmental processes that are important for normal brain development in rodents (upper panel) and humans (lower panel) are shown. Birth (B), weaning (W), puberty (P) and adulthood are indicated separately in each panel for the rodent or human development. The different timescales used across species (23 days in rodents, 9 months in human) highlight the significant differences in the speed of maturation across species. The time of brain growth spurt in humans (full term birth) and rodents [around postnatal day (PN) 10] has been used to indicate the ages across species that correspond to a full term newborn human baby
Brain growth spurt in these studies included gross brain growth, DNA, cholesterol, and water content. Puberty onset occurs around PN32-36 in female rats and PN35-45 in male rats, whereas in humans it starts around 10-11 years in girls and 11-12 years in boys (92). Distinct processes, such as neurogenesis and migration, synaptogenesis and synaptic pruning, myelination follow different time courses (90, 93–100). However, the staging of the equivalence of developmental stages across species is only approximate and each developmental process needs to be considered individually. B. Significant changes occur during development in the expression or function or various signaling processes. A schematic depiction of the age-related changes in GABA<sub>A</sub>R and glutamatergic signaling in rats is presented here, however cell type, regional and sex-specific differences also exist (90, 105, 622). Early in development, there is less effective GABA<sub>A</sub>R-mediated inhibition, due to the presence of depolarizing GABA<sub>A</sub>R signaling (see also FIGURE 4), more tonic and less phasic GABA<sub>A</sub>R inhibition. In contrast, glutamatergic receptors, such as NMDAR or kainate receptors also show age-related expression patterns.

**FIGURE 4. Depolarizing and hyperpolarizing GABA<sub>A</sub>R signaling in normal development and disease.** A. GABA<sub>A</sub>R signaling is depolarizing early in life due to the higher intracellular Cl- concentrations ([Cl<sup>-</sup>]<sub>i</sub>) that force Cl- efflux upon GABA<sub>A</sub>R activation. Although the GABA<sub>A</sub>R depolarizations render GABA inhibition less efficient, as it relies upon shunt inhibition, they are critical for normal brain development. GABA<sub>A</sub>R depolarizations may activate L-type voltage sensitive calcium channels and may release the Mg<sup>++</sup> block of NMDARs, triggering intracellular Ca<sup>++</sup> rises that are important for neuronal survival, migration, differentiation and integration (123, 130, 132, 623). The [Cl<sup>-</sup>]<sub>i</sub> in immature neurons is a result of increased expression and/or activity of Cl<sup>-</sup> importers, like NKCC1 (a Na<sup>+</sup>/K<sup>+</sup>/Cl<sup>-</sup> cotransporter) over Cl<sup>-</sup> exporters, like KCC2 (a K<sup>-</sup>/Cl<sup>-</sup> cotransporter). The Na<sup>+</sup>/K<sup>+</sup> ATPase provides the energy to maintain the cation chloride
cotransporter function. During development, there is a gradual switch in the relative dominance of these cation chloride cotransporters at specific timepoints that follows cell type, region, and sex specific patterns (108, 122, 125, 126). As a result, mature neurons demonstrate hyperpolarizing GABA$_A$R responses that allow for effective inhibition to occur. B. Normal brain development depends upon the age, cell type, region, and sex appropriate presence of depolarizing and hyperpolarizing GABA$_A$R signaling. Genetic variants, drugs, perinatal or postnatal insults that trigger precocious presence of hyperpolarizing GABA$_A$R signaling may result in neurodevelopmental deficits or abnormalities that could increase the risk for epilepsy (130, 132, 623). Conversely, pathological persistence or re-appearance of depolarizing GABA has been described in epileptogenic pathologies and may predispose to increase excitability (133).

**FIGURE 5. Simplified diagram of a cortical microcircuit with interconnected Glutamatergic and GABAergic neurons, and an astrocyte, and cellular/subcellular distribution of ion channels and transporters.** A cortical neuronal microcircuit is illustrated as a pre-synaptic GABAergic neuron (green) and a presynaptic myelinated glutamatergic neuron (ocher) that form synaptic connections on the dendrites of a myelinated glutamatergic neuron (ocher). Glial cells are displayed as an astrocyte (light blue) in proximity of the glutamatergic synapses, and as the myelin sheets around the axons of the glutamatergic neurons formed by oligodendrocytes (violet; the soma is not displayed), allowing saltatory conduction at the nodes of Ranvier. The upper insets show in more detail a GABAergic (left) and a glutamatergic (right) synapse. The ion channels and transporters targeted by DEE mutations are indicated with their protein name (see text for details) and their known cellular/subcellular distribution, according to the neuronal sub-compartment (dendrites, soma, axon initial segment, nodes of Ranvier of the myelinated axon, pre-synaptic terminal, and post-synaptic membrane).
FIGURE 6. Schematic representation of the different in vitro and in vivo models that can be used to study functional effects of mutations affecting DEEs causative genes. Regardless of the starting point, researchers can move from a model to another based on the type of functional assay they want to apply and the physiological process they want to study. EPS: electrophysiological studies; MRI: magnetic resonance imaging; BEH: behavioral studies; ICC: immunocytochemistry; LI: live imaging; TR: transcriptomics; PR: proteomics; IHC: immunohistochemistry; ISH: in situ hybridization.

FIGURE 7. Brain MRI of patients with different malformations of cortical development. A. T1-weighted (T1W) coronal section. Lissencephaly in a boy with ARX mutation. The ventricles are severely dilated, the corpus callosum is absent, the basal ganglia are severely hypoplastic. B, C. Coronal T1W and Axial T2W sections of a brain with posterior > anterior pachygyria and increased cortical thickness. Boy with LIS1 mutation. The white asterisk in B indicates the point of more severe cortical thickening. White arrows in C point to areas of more severely smooth and thick cortex. D. T1W coronal section. Diffuse subcortical band heterotopia in a girl with DCX mutation. The white circle surrounds the subcortical laminar heterotopia which forms an almost continuous band beneath the cortex, separated from it by white matter. E. Axial T2W section. Right occipital cortical dysplasia (surrounded by a white circle) in a girl with a very low-level mosaic mutation in AKT3 (0.67% in brain, not detectable in blood). F, G. Axial FLAIR and coronal T1W sections in two patients carrying mosaic mutations in the MTOR gene with different percentages of mosaicism [F: p.Thr1977Ile, 20% of mosaicism in blood, G: p.Ser2215Phe, 5.5% of mosaicism in the surgically removed dysplastic brain tissue]. In F, the patient has megalencephaly with large ventricles and multiple areas of abnormal cortex alternating infoldings with smooth surface. This pattern is suggestive of polymicrogyria (white arrows). In G, the white circle highlights an area of cortical
dysplasia with increased volume of the brain parenchyma, blurring of the gray-white matter junction and irregular cortical folding. **H.** T2W coronal section. Left parieto-temporal focal cortical dysplasia in a girl with *NPRL2* mutation. The circle surrounds the parietal portion of the cortical abnormality. **I, J.** T1W axial sections in two patients carrying the p.Gly373Arg *PIK3R2* gene mutation with different percentages of mosaicism (I: 13% of mosaicism in blood, 43% in saliva, J: 10% of mosaicism in blood, 29% in saliva). Both patients have bilateral perisylvian polymicrogyria (white circles). **K, L.** Axial FLAIR and coronal T1W sections showing right posterior quadrantic dysplasia (white circle) in a boy with a constitutional *PTEN* mutation. **M, N.** T2W coronal and sagittal sections in two patients with constitutional *TSC2* mutations (**M**: p.Thr1623Ile, **N**: p.Pro1202His) showing right posterior quadrantic dysplasia caused by a large cortical tuber (**M**, white circle) and an extensive dysplastic area involving most of the right frontal lobe (**N**, white arrowheads). **O, P.** T2W axial and T1W sagittal sections. Lissencephaly with normally thick cortex and cerebellar hypoplasia (**P**, asterisk) in a girl with *RELN* mutation. The white circle surrounds a hypoplastic brainstem. **Q, R.** Axial and sagittal T1W sections. Thickened cortex with simplified gyral pattern and cerebellar hypoplasia in a boy with *TUBA1A* mutation. The circles surround the hypoplastic cerebellum and brainstem. The asterisk indicates the area below a hypoplastic cerebellar vermis and the black arrow points to a hypoplastic corpus callosum lacking its most posterior part. **S.** T1W axial section. Diffusely simplified gyral pattern with prominent thickening and infolding of the sylvian fissures in a boy with *TUBB2B* mutation. The arrows point to an area of smooth cortex. **T.** T2W axial section. Severe dysgyria with simplified gyral pattern in a girl with *SCN3A* mutation. **U.** T1W axial section. Classical bilateral periventricular nodular heterotopia in a girl with *FLNA* mutation. Bilateral nodules of subependymal heterotopia (white arrowheads) are contiguous, extensively lining the ventricular walls. **V.** T1W axial section. Diffuse polymicrogyria, more prominent posteriorly (white arrows) in a boy with *ATP1A2* mutation. **W.** T2W coronal
Polymicrogyria with abnormal cortical infoldings and packed microgyria (black arrows), combined with abnormal sulcation in a boy with ATP1A3 mutation. X. T2W axial section. Bilateral frontoparietal cortical thickening and diffusely abnormal cortical pattern in a boy with biallelic GPR56 mutations. Y, Z. T1W axial and sagittal sections. Pachygyria and perisylvian polymicrogyria in a girl with DYNC1H1 mutation. Asterisks in Y are located where there is maximum cortical thickening, in the posterior cortex. The asterisk in Z is located beneath a hypoplastic cerebellar vermis. AA. T2W axial section. Diffuse polymicrogyria in a boy with a GRIN2B mutation. BB. T1W axial section. Diffuse abnormality of the cortical pattern with smooth cortex and areas of abnormal infolding, suggestive of polymicrogyria in a boy with biallelic WDR62 mutations.

FIGURE 8. Main actors of synaptic transmission and mapping of the synaptic gene products causing synaptic encephalopathies with epilepsy. Schematic representation of a symbolic synapse containing excitatory and inhibitory synaptic components. The main targets of synaptopathies are: (i) at the presynaptic level, gene products involved in the post-docking SV priming/fusion processes (SNAREs and SNARE-associated proteins: Munc-13, IM1, Munc-18, PRRT2, SNARE proteins, synaptotagmin-1/2, voltage-gated Ca^{2+}-channels), SV trafficking (trafficking proteins: Synapsins I/II, vATPase, synaptophysin, SV2A, VAMP2, synaptojanin-1, AP-2, dynamin-1, TBC1D24) and NT synthesis and loading into SVs (transport proteins: GAD1, vATPase); (ii) at the postsynaptic level, postsynaptic receptors and their scaffold/transduction systems (GABA_A and NMDA receptors, gephyrin, collybistin, PSD-95, Homer, Shank-3, SynGAP-1, DLG-1); (iii) at the synaptic cleft level, trans-synaptic and extracellular matrix proteins and their receptors (neurexin-1, neuroligin, IL1RAPL1, ADAM 22/23, LGI1), as well as secreted proteins (SRPX2, reelin). Other presynaptic voltage-gated channels that affect the dynamics of nerve terminal activation and NT release are also shown. In green, the actin-based cytoskeleton that regulates trafficking and...
maintenance of SV pool in the nerve terminal and concentrates postsynaptic receptors on the
postsynaptic side.

**FIGURE 9. Schematic representation of the mTOR and autophagy intracellular cascades and their
interrelationships.** The complex regulatory cascade triggering the activation of the mTORC1
complex is initiated by extracellular signals (growth factors, neurotransmitter, hormones). To be
activated, mTORC1 needs to bind to the organelle membrane, a process that depends on the
active form of the small G-protein Rheb and by the presence of a docking complex on the
membrane formed by the GEF Ragulator and an appropriate combination of GTP- and GDP-bound
Rag G-proteins. The membrane location of the latter complex depends on the presence of the
vATPase on the membrane, which is favored by Dmxl2. Since the small G-proteins are the
molecular switches for mTOR activation, they are also the targets of the two main upstream
inhibitory complexes that act as GTPase activating proteins (GAPs), namely the TSC and Gator1
complexes that inactivate Rheb and Rags, respectively. These inhibitory TSC and Gator1 complexes
are in turn subjected to inhibition by the PI3K/AKT pathway, activated by extracellular signals and
Gator 2, respectively, that therefore catalyze release of mTORC1 from inhibition. Activation of
mTORC1 favors anabolism, protein synthesis, cell growth and, in neurons, outgrowth of neuronal
processes and formation of synaptic connections. On the other hand, activation of mTORC1
silences the autophagy chain by inhibiting the ULK1 complex which is required to recruit the Beclin
complex to the phagophore for its activation by AMPK. This results in the following steps of
autophagy flux, including LC3 conversion and binding, formation of the autophagosome and
subsequent fusion with lysosomes to form autolysosomes. In these processes, the proton gradient
established by vATPase is essential, as well as the activity of accessory proteins such as DMXL2,
EPG5 and SNX14.
A

| 1 | 2 | 3 | 4 | 5 | 6 | P | 7 | 8 | 9 |

↓ CANONICAL SPLICING

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

↓ TRANSLATION INTO THE WILD TYPE PROTEIN

INTEGRATION INTO THE PLASMA MEMBRANE AND NORMAL FUNCTIONING

B

| 1 | 2 | 3 | 4 | 5 | 6 | P | 7 | 8 | 9 |

↓ ABERRANT SPLICING

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

↓ TRANSLATION INTO A TRUNCATED PROTEIN

RECOGNITION OF A PTC AND DEGRADATION OF TRUNCATED PROTEIN
A

Rodents

- 23 days (rodents)
- Synaptic pruning
- Synaptogenesis
- Neurogenesis, migration
- Myelination
- Brain growth spurt

- Birth (B)
- Weaning (W)
- Puberty (P)

- 9 months (humans)

- Adulthood

Human

- Brain growth spurt
- Myelination
- Neurogenesis, migration
- Synaptogenesis
- Synaptic pruning

B

Developmental changes in GABA/ glutamate receptor signaling in rodents

- Birth
- Weaning
- Puberty

- Phasic GABA\(_A\)R signaling
  - hyperpolarizing
  - depolarizing

- Tonic GABA\(_A\)R signaling
- NMDAR signaling
- Kainate receptors signaling

Age (postnatal weeks): 0 1 2 3 4 5 6 7
**A**

Immature neurons, High intracellular [Cl-]

- GABAAR
- NMDAR
- KCC2
- NKCC1

Mature neurons, Low intracellular [Cl-]

- GABAAR
- NMDAR
- KCC2
- NKCC1

Depolarization
- DNA synthesis
- Proliferation
- Migration
- Survival
- Synaptogenesis
- Neuronal differentiation

Hyperpolarization
- Increased excitability (??)
- Shunt inhibition
- Effective inhibition

**B**

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<th>Hyperpolarizing GABA</th>
<th>Normal brain development and sexual differentiation</th>
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Genetic variants, Drugs, Perinatal/postnatal insults

Pathological persistence / re-appearance of depolarizing GABA

Neurotrophic effects

Increased excitability, Epilepsy, Epileptogenic lesions / malformations

Neurodevelopmental abnormalities and disorders

Precocious appearance of hyperpolarizing GABA

No epilepsy