Developmental and epileptic encephalopathies: from genetic heterogeneity to phenotypic continuum

Renzo Guerrini, Valerio Conti, Massimo Mantegazza, Simona Balestrini, Aristea S Galanopoulou, Fabio Benfenati

Developmental and epileptic encephalopathies: from genetic heterogeneity to phenotypic *continuum*

Renzo Guerrini[1,2], Valerio Conti[1], Massimo Mantegazza[3-5], Simona Balestrini[1,2], Aristea S Galanopoulou[6,7] and Fabio Benfenati[8,9]

[1]Neuroscience Department, Meyer Children's Hospital, Florence, Italy

[2]University of Florence, Florence, Italy

[3]Université Côte d'Azur, CNRS UMR727, Valbonne-Sophia Antipolis, France.

[4]CNRS UMR7275, Institute of Molecular and Cellular Pharmacology (IPMC), Valbonne-Sophia Antipolis, France.

[5]Inserm, Valbonne-Sophia Antipolis, France.

[6]Saul R. Korey Department of Neurology, Isabelle Rapin Division of Child Neurology, Albert Einstein College of Medicine, Bronx NY USA.

[7]Dominick P Purpura Department of Neuroscience. Albert Einstein College of Medicine, Bronx NY USA.

[8]Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia, Genoa, Italy.

[9]IRCCS Ospedale Policlinico San Martino, Genoa, Italy.

Short title: Developmental and Epileptic Encephalopathies

Address for reprint requests and other correspondence:

Prof. R. Guerrini, Pediatric Neurology, Neurogenetics and Neurobiology Unit and Laboratories, Meyer Children's Hospital, Viale Pieraccini 24, 50139 Florence, Italy (e-mail: r.guerrini@meyer.it, phone: +390555662573, fax: +390555662329).

Table of Contents

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

(ILAE) Task Force on Nosology and Definitions (5–7). 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 *Scn1a*-R1648H allele with either $Scn2a^{Q54}$, which causes spontaneous, adult-

onset partial motor seizures or *Kcnq2*$^{V182M/+}$, which causes increased susceptibility to induced seizures. 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 $Na_V1.1$ 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 $Na_v1.1^{+/-}$ mice with targeted deletion of the last encoding exon, than the 129/SvJ background (70). In the C57BL/6 background, $Na_v1.1^{+/-}$ 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 *in vivo* and *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-$43^{Cx43fl/fl:hGFAP-Cre}$) 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 *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 ($GABA_AR$ and $GABA_BR$ 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 ($GABA_AR$) and include changes in receptor composition and kinetics of, depolarizing/hyperpolarizing postsynaptic $GABA_A$ receptor ($GABA_AR$) responses or network effects (105–111) (**FIGURE 4**). $GABA_AR$ 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 $GABA_AR$ 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 $GABA_AR$ 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 temporo-spatial pattern of maturation not only of $GABA_ARs$, but also of other neurotransmitter and signaling pathways. For example, hyperpolarizing $GABA_AR$ responses may occur earlier in rat CA1 pyramidal neurons than in substantia nigra pars reticulata GABAergic neurons (111, 126); $GABA_AR$ mediated hyperpolarizing responses are already seen in neonatal thalamic neurons, in contrast to cortical

neurons which mature later on (127). The maturation to hyperpolarizing $GABA_AR$ 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 $GABA_AR$ signaling may disrupt the excitatory synapse formation and dendritic arborization of cortical neurons, leading to neurodevelopmental deficits (130–132). Disruptions of the $GABA_AR$-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 $GABA_ARs$ in the SNR exerts anticonvulsant effects in a variety of seizure models (143–145). However, in developing rodents, the outcome of $GABA_AR$-sensitive SNR-mediated seizure control (anticonvulsant vs proconvulsant

vs no effect) varies by region, age, and sex (110, 141, 142). All this knowledge remains too fragmentary, but it represents a promising field to fully understand the human pathology.

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 sequalae 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 $GABA_AR\gamma2$(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

592 circumstances, would participate to the formation of the normal cerebral cortex, and seizures can
593 arise because of neuronal malpositioning, abnormal proliferation or differentiation, or abnormal
594 cortical organization (186).
595 Neuronal malpositioning is the result of altered neuronal migration during brain development. A
596 classic example of DEE associated with neuronal migration deficit is caused by mutations in the
597 aristaless-related homeobox (*ARX*) gene, located on chromosome Xp21, which can result in a
598 phenotypic spectrum comprising a nearly continuous series of neurodevelopmental disorders
599 including lissencephaly with ambiguous genitalia (XLAG) (**FIGURE 7A**), Proud syndrome, Partington
600 syndrome, infantile spasms without brain malformations, and syndromic and non-syndromic
601 intellectual disability with epilepsy.
602 The ARX protein belongs to the Aristaless-related subset of the paired (Prd) class of homeodomain
603 proteins. Homeodomain transcription factors play crucial roles in cerebral development and
604 patterning (187). In particular, ARX is involved in the normal tangential migration of GABAergic
605 neurons and the occurrence of seizures in most patients carrying mutations in this gene can be
606 ascribed to mislocalization or malfunction of this class of neurons and loss of inhibitory
607 neurotransmission (188). For this reason, *ARX*-related DEEs is considered a "developmental
608 interneuronopathy", a term coined to differentiate developmental brain disorders caused by
609 impaired development, migration, or function of interneurons from functional deficits or
610 secondary loss of interneurons and from the more common channelopathies (189).
611 Developmental interneuronopathies may also include Dravet syndrome, as discussed above, and
612 classical lissencephaly due to mutations in *PAFAH1B1*, also known as *LIS1* (**FIGURE 7B, C**), or *DCX*
613 (**FIGURE 7D**). The *PAFAH1B1* gene encodes for the regulatory beta-subunit of the cytosolic type I
614 platelet-activating factor (PAF) acetylhydrolase, which is involved in interneuronal migration and
615 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

640 normal-appearing glia or abnormal reactive astrocytes, which overexpress specific intermediate
641 filaments and other immature molecular markers. Taken together, these findings strongly
642 implicate primary defects in cellular proliferation and differentiation in the pathogenesis of
643 mTORopathies (192).
644 Electrophysiological recordings in neocortical samples surgically removed from patients with FCD
645 have demonstrated that, under reduced K^+ conductance, dysmorphic cytomegalic neurons, but
646 not balloon cells, generate large Ca^{2+} currents when stimulated, indicating that this aberrant cell
647 type plays an important role in epileptogenesis (193). Both the morphology and size of dysmorphic
648 cytomegalic neurons have been implicated with the origin of aberrant electrical discharges. By
649 using electrophysiology, calcium imaging, morphological analyses, and modeling studies, Williams
650 and collaborators demonstrated that *Pten* (an mTOR pathway inhibitor) knock-out neurons exhibit
651 rapid-onset hypertrophy and higher density of synapses proximal to the soma and that these
652 phenotypic abnormalities promote firing at more hyperpolarized membrane potentials, with
653 greater peak spike rates, and higher sensitivity to depolarizing synaptic input (194).
654 Using mouse models with cell-type-specific mTOR pathway activation obtained by electroporating
655 the *Pik3ca* p.H1047R mutation, which is associated with both FCD and HME, D'Gama and
656 collaborators demonstrated that mTOR pathway activation in excitatory neurons and glia, but not
657 in interneurons, is sufficient to cause abnormal cortical lamination and overgrowth (195). In FCD,
658 epileptic seizures appear to be triggered by a peculiar mechanism which we detail below. By
659 treating *in vitro*-maintained organotypic slice cultures of resected FCD lesions with 4-
660 aminopyridine, a potent convulsant agent acting as potassium channel blocker, Avoli and
661 collaborators demonstrated intrinsically generated ictal-like epileptiform events (196). These
662 epileptiform events were triggered by an amino acid receptor-mediated mechanism which was
663 glutamatergic-independent and mainly attributable to a $GABA_A$ receptor-mediated conductance

(196). 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). Na_V are composed by a principal pore-forming α subunit (nine isoforms: $Na_V1.1$-$Na_V1.9$ 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 (DI-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. *SCNs*/Na_Vs are targets of clinically used sodium channel blockers, among them several anti-seizure medications (ASMs) (202). Mutations in *SCN1A*/$Na_V1.1$, *SCN2A*/$Na_V1.1$, *SCN3A*/$Na_V1.1$ and *SCN8A*/$Na_V1.6$ 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).

688 **1.1. $Na_V1.1$ channels (*SCN1A*)**

689 *SCN1A*, encoding the $Na_V1.1$ α subunit, is one of the most clinically relevant epilepsy genes, with
690 hundreds of mutations reported thus far, whose associated phenotypes range from Dravet
691 syndrome, a severe form of DEE, to milder GEFS+ and other diseases, such as hemiplegic migraine
692 (4, 209, 210).

693 Dravet syndrome is caused by often *de novo* heterozygous *SCN1A* mutations (211, 212), of which
694 approximately half are missense, and half are predicted to give rise to a truncated non-functional
695 protein. The clinical spectrum of Dravet syndrome does not have firmly established boundaries,
696 but the core phenotype is characterized by intractable seizures precipitated by increased body
697 temperature with onset between six months and one year of age, and subsequent appearance of
698 multiple hyperthermia-induced and hyperthermia-independent seizures. Development is normal
699 in the first year of life but plateaus rapidly, with most patients showing cognitive impairment. In
700 patients with Dravet syndrome, *SCN1A* mutations can also be inherited from a parent with less
701 severe clinical manifestations, at times carrying somatic mosaicism (213). It has been proposed
702 that mutations in other genes can cause Dravet syndrome-like phenotypes (including *SCN1B*,
703 *HCN1*, *KCN2A*, *GABRA1*, *GABRG2*, and *STXBP1*), but with a specific clinical pictures (see below).
704 Thus, the association between *SCN1A* mutations and Dravet syndrome is highly specific. The
705 familial epilepsy GEFS+ syndrome can be also caused by heterozygous missense *SCN1A* mutations
706 and is characterized by febrile seizures plus (FS+: febrile/hyperthermic seizures that extend
707 beyond six years of age) and afebrile generalized tonic-clonic seizures (GTCS), at times including
708 absence, myoclonic, atonic, or focal seizures, and Dravet syndrome. Missense *SCN1A* mutations
709 can also cause sporadic/familial hemiplegic migraine type3 (S/FHM3), a rare form of migraine with
710 aura and onset in adolescence, characterized by hemiparesis as part of the aura phase. Moreover,
711 two missense mutations with gain of function have been associated with an extremely severe

712 early infantile DEE with earlier seizure onset, profound cognitive and motor impairments, and a
713 hyperkinetic movement disorder (214–216).
714 The functional effect of truncating mutations causing Dravet syndrome has since their
715 identification thought to be haploinsufficiency: 50% reduction of functional $Na_V1.1$ protein in
716 heterozygotes, with complete loss-of-function (LoF) (211), which has been verified in functional
717 studies (217). The effect of missense mutations studied in transfected cell lines has been more
718 controversial, but most of the results point to a LoF, whose severity tends to correlate with that of
719 the phenotype (199, 218). Thus, the severity spectrum of *SCN1A*-related epilepsies could be a
720 continuum and depend on the amount of LoF of the mutant. Some *SCN1A* missense mutations
721 cause LoF because of folding/trafficking defects that lead to channel degradation (219) that can at
722 least partially be rescued by interacting proteins stabilizing the correct folding conformation (220–
723 223).
724 $Na_V1.1$ is the predominant Na^+ channel of GABAergic interneurons whose decreased excitability
725 induced by *SCN1A* epileptogenic mutations reduces GABAergic inhibition and causes network
726 hyperexcitability (64). A study with a knock-in model expressing a truncating nonsense DS
727 mutation reported a similar phenotype, showing that $Na_v1.1$ localizes to the axon initial segment
728 of GABAergic interneurons, in particular fast-spiking parvalbumin (PV)–positive ones (65).
729 Subsequent studies have shown that these mice display also co-morbidities including cognitive
730 and behavioral deficits, ataxia, SUDEP, dysregulated circadian rhythms and sleep dysfunctions
731 (199, 218, 224, 225). Several other studies, including those performed with conditional mouse
732 models that expresses the mutations in specific neuronal subtypes (199, 218) and with a knock-in
733 model of a GEFS+ mutation (*Scn1a*$^{R1648H/+}$) (226, 227) have confirmed that hypoexcitability of
734 GABAergic neurons is the initial pathological mechanism in Dravet syndrome models and that the
735 amount of LoF can determine the severity of the phenotype. Notably, *Scn1a* mouse models have

736 also allowed to disclose genetic modifiers and cellular remodeling induced by the initial effect of
737 the *Scn1a* mutation, which can modulate the phenotype implementing homeostatic or pro-
738 pathologic modifications, including homeostatic upregulation of Na^+ channels in GABAergic
739 neurons and seizure-induced hyperexcitability of glutamatergic neurons (199, 218). For instance,
740 the interaction between seizures and the genetic mutation can induce remodeling in the
741 *Scn1a*$^{R1648H/+}$ mouse model transforming a basically asymptomatic phenotype into a Dravet
742 syndrome-like one, because of increased excitability of excitatory glutamatergic neurons (228).
743 Overall, these results show that the initial pathological mechanism in mouse models of human
744 epileptogenic *SCN1A* mutations is LoF and hypoexcitability of at least some subtypes of GABAergic
745 neurons. However, this initial dysfunction triggers both homeostatic and pathologic remodeling,
746 depending on the neuronal types, age, genetic background, and interactions between *SCN1A*
747 mutations and experienced seizures.
748 Although some drugs like stiripentol (229), fenfluramine (230) or cannabidiol (231) have been
749 shown to be partially effective in some patients, Dravet syndrome still remains drug-resistant for
750 most of the patients. *Scn1a* gene targeted mice have been used for testing therapeutic
751 approaches, both classical pharmacological treatments and novel methods, including gene
752 therapy, in some cases obtaining significant amelioration of the phenotype (199, 232). Some novel
753 approaches have shown particularly effective results and have led to clinical trials. For example, an
754 antisense oligonucleotide-based targeted augmentation of nuclear gene output (TANGO)
755 approach has been used to increase the expression of functional $Na_V1.1$ channels in Dravet
756 syndrome mice, observing a substantial decrease in spontaneous seizures and SUDEP when
757 specific antisense oligonucleotides were injected in newborn mice (233).
758 Mutations causing sporadic/familial hemiplegic migraine (S/FHM) cause $Na_V1.1$ gain-of-function
759 (GoF) by inducing gating modifications and increasing the slowly inactivating “persistent” Na^+

760 current (199, 218). Gene targeted mouse models of S/FHM mutations have been generated and
761 they show facilitated/spontaneous generation of cortical spreading depression (CSD), a proposed
762 pathological mechanism of migraine with aura, but no seizures (234, 235). Genetic and acute
763 models have shown that these mutations induce hyperexcitability of GABAergic neurons leading to
764 increase of extracellular K^+ and clamped depolarizing block of neuronal activity (236). A very mild
765 GoF has also been observed for the p.T226M mutation causing an extremely severe early infantile
766 *SCN1A* DEE (216), but it is not clear yet how such a mild change could induce the severe
767 phenotype.

768 **1.2. $Na_V1.2$ channels (*SCN2A*)**

769 *SCN2A* encodes the $Na_V1.2$ sodium channel, which is widely expressed in the central nervous
770 system, particularly in cortical and hippocampal glutamatergic neurons (199). These are the main
771 Na^+ channels of the axonal initial segment (AIS) and the nodes of Ranvier in the first 10 days of
772 postnatal life in rodents, then they are partially replaced by *SCN8A*/$NA_V1.6$. It is thought that
773 $Na_V1.2$ channels in the proximal AIS promote backpropagation to the soma. In the adult brain,
774 $Na_V1.2$ is also present in thin processes, presumably distal unmyelinated portions of preterminal
775 axons.

776 The first epilepsy syndrome clearly associated with *SCN2A* mutations was benign familial
777 neonatal/infantile seizures (BFNIS) (237), characterized by a mild phenotype. Subsequently, it has
778 been shown that mutations in *SCN2A* cause a wide range of neurodevelopmental disorders,
779 including DEE of varying severity. DEE mutations generally arise *de novo*, and about 80% are
780 missense. About 60% of *SCN2A* DEE have onset in the first three months of life (238, 239). All
781 neonatal-early infantile DEE patients display intellectual disability. About 50% of them have
782 variable seizure types, whereas the others have Ohtahara syndrome (neonatal-onset spasms or
783 tonic seizures and EEG with burst suppression pattern), sometimes evolving into West syndrome,

784 Lennox–Gastaut phenotypes or epilepsy with early infantile migrating focal seizures (EIMFS). DEE
785 with infantile and childhood onset are about 40% of the total (238, 239). The phenotype can be
786 correlated to the age of onset. Patients with onset between three months and one year of age in
787 general have West syndrome, with possible evolution into a Lennox–Gastaut phenotype. Patients
788 with onset after one year of age often show variable seizure phenotypes with
789 developmental/cognitive delay and autistic traits that can appear before seizure onset.
790 Functional analysis in transfected cell lines has shown some genotype-phenotype correlations
791 (199, 240). GoF mutations are related to mild benign neonatal/infantile epilepsy and to
792 neonatal/early infantile DEE, which can be responsive to treatment with Na^+ channel blockers
793 (239), whereas LoF mutations are linked to infantile/childhood epileptic encephalopathy or
794 neurodevelopmental disorders (autism and intellectual disability) without seizures. However,
795 identifying genotype-phenotype correlations within these GoF and LoF mutants is difficult due to
796 partially overlapping phenotypes.
797 Recordings in brain slices of heterozygous knock-out *Scn2a* (*Scn2a*$^{+/-}$) mice have shown that
798 $Na_v1.2$, besides its established axonal role, has important dendritic functions in pyramidal neurons
799 of the prefrontal cortex, where its haploinsufficiency impairs synaptic plasticity and synaptic
800 strength, even when *Scn2a* expression is reduced in single neurons late in postnatal development
801 (241). However, these studies have shown that *Scn2a*$^{+/-}$ mice, differently than *Scn1a* models, have
802 a relatively mild phenotype, including short absence-like seizures, spatial memory deficits but
803 enhanced fear memory, plus autistic traits (199, 242). A *Scn2a* mouse model carrying the severe
804 hypomorphic mutation Δ1898 shows a more severe phenotype, with robust autistic-like features
805 (243). Two additional studies have shown that either complete deletion (244) or reduced
806 expression of *Scn2a* (244, 245) can paradoxically induce hyperexcitability of glutamatergic
807 neurons. Overall, these findings suggest that reduction of *SCN2A* to more than haploinsufficiency

808 could be needed to induce severe phenotypes. A knock-in mouse model of the recurrent GOF
809 mutation R1882Q has been generated (246) showing that heterozygous mice display
810 hyperexcitability of cortical pyramidal neurons, and develop spontaneous seizures at P1 and
811 premature death between P13 and P30. Interestingly, reduction of $Na_V1.2$ expression by specific
812 antisense oligonucleotides reduced seizures and extended lifespan.

813 **1.3. $Na_V1.3$ channels (*SCN3A*)**

814 *SCN3A* encodes the $Na_V1.3$ Na^+ channel, whose functions have not been completely determined
815 yet. *SCN3A* is widely expressed in the brain at high levels during embryonic development and is
816 downregulated afterwards (199).

817 *SCN3A*-related clinical phenotypes comprise a wide spectrum, including mild epilepsy with
818 intellectual dysfunction, early infantile DEE often associated with polymicrogyria, as well as speech
819 and oral motor dysfunctions associated with polymicrogyria without epilepsy. Relatively mild
820 neonatal-childhood onset focal epilepsy (247), would be the mildest phenotype in the spectrum
821 (248, 249). Disrupted cerebral cortical folding and neuronal migration were recapitulated in ferrets
822 expressing the mutant *SCN3A* (249). Functional studies in transfected human cell lines have shown
823 that most of the *SCN3A* mutations associated with severe human phenotypes exhibit prominent
824 GoF, inducing, in particular, large increases of persistent Na^+ current (248–250). LoF caused by
825 reduced current density has been reported for some *SCN3A* variants. Heterozygous adult *Scn3a*$^{+/-}$
826 knock out mice were investigated as a model of *SCN3A* LoF mutations, but they do not show
827 features of DEE (251).

828 **1.4. $Na_V1.6$ channels (*SCN8A*)**

829 *SCN8A* encodes $Na_V1.6$, the main Na^+ channel in the axon initial segment and nodes of Ranvier of
830 myelinated axons in mature excitatory neurons (199).

831 *De novo* heterozygous mutations in *SCN8A* have been identified in a range of phenotypes. Most
832 patients show a DEE with multiple seizure types and early onset, severe intellectual disability and
833 movement disorders and have an increased risk of SUDEP (252). Other patients exhibit milder
834 phenotypes, including benign familial infantile seizures (BFIS) and epilepsies with intermediate
835 phenotypes, or generalized epilepsy with absence seizures (253, 254). Some patients show
836 intellectual disability, autism, or movement disorders without epilepsy (255, 256). About 20% of
837 patients have recurrent mutations.
838 Mutations that cause DEE or milder epilepsy induce GoF with negative shifts of voltage-
839 dependence of activation, positive shifts of voltage-dependence of inactivation, slowed channel
840 inactivation, or increased persistent or resurgent current. These functional changes are all
841 consistent with neuronal hyperexcitability, which has been confirmed in transfected cultured
842 neurons (255, 257). Other mutations causing intellectual disability, autism, or movement disorders
843 without epilepsy induce LoF, which can be complete (255, 256). Massive GoF can induce reduced
844 generation of APs, mimicking LoF mutations (255). LoF mutations have been identified in patients
845 with absence seizures (254), a phenotype reproduced by heterozygous loss of function mouse
846 models.
847 A standard global knock-in (258) and a conditional floxed knock-in (259) mouse models of *SCN8A*
848 DEE mutations are available. Overall, knock-in mice indicate that *SCN8A* GoF mutations are
849 sufficient to induce hyperexcitability of some subtypes of excitatory neurons, generating severe
850 seizures and a lethal phenotype. In contrast, spontaneous mouse models carrying LoF *SCN8A*
851 mutations (260) show a phenotype similar to that of patients with intellectual disability and/or
852 movement disorders without epilepsy.
853 Some patients with *SCN8A* GoF mutations respond to high doses of sodium channel blockers (254,
854 261), 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

879 fast-spiking interneurons. GABAergic interneurons were hypoexcitable, whereas dysfunctions of
880 pyramidal neurons were more complex, with subsets of them exhibiting hyperexcitability at low
881 current injections, as well as hypoexcitability at high stimulation intensities.

882 **2. VOLTAGE-GATED K^+ CHANNELS**

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

890 **2.1. M-current K^+ channels (K_V7, *KCNQ*)**

891 Five *KCNQ* genes have been identified that encode K_V7 K^+ channels, which can form both homo-
892 and hetero-tetramers (267, 268). Of the four channels expressed in the nervous system (*KCNQ2-*
893 *5*), three (particularly *KCNQ2,* but also *KCNQ3* and *KCNQ5*) host DEE mutations. *KCNQ2* and *KCNQ*3
894 are expressed in many brain areas, whereas expression of *KCNQ*5 is more limited (269). They
895 generate the M (muscarinic receptor inhibited)-current, a slow non-inactivating K^+ current that
896 activates at subthreshold membrane potentials (270). *KCNQ2*-linked DEE has its onset in early
897 infancy and features motor impairment and variable intellectual disability (271). Often, the EEG
898 initially shows a burst suppression pattern that may later evolve to multifocal epileptiform activity.
899 *KCNQ3* and *KCNQ5* DEEs display a variable degree of severity and some patients show intellectual
900 disability apparently without epilepsy (9, 267). Mutations in the three *KCNQ* genes can also cause
901 “benign familial neonatal seizures” (BFNS), which is mild and self-limited (268).

902 According to their subcellular localization, K_V channels can regulate specific features of neuronal
903 excitability. Somato-dendritic channels are strongly activated by back-propagating APs, attenuate
904 repetitive firing and contribute to the medium and slow components of the afterhyperpolarization
905 (AHP), determining the refractory period and regulating spiking frequency. In the perisomatic
906 region, slow activation of the M current decreases neuronal firing in response to sustained stimuli,
907 inducing spike-frequency adaptation (269, 272, 273). Axonal K_V channels mostly function by
908 stabilizing the resting membrane potential, leading to increased activation of axonal Na_V channels
909 (274–276) and of presynaptic Ca^{2+} channels, modulating synaptic release (277, 278). Both *KCNQ2*
910 and *KNCQ3* are also expressed in GABAergic interneurons (279).
911 Numerous *in vitro* studies have investigated functional effects of *KCNQ* DEE mutations in cell lines
912 or *Xenopus* oocytes (267). Most studies agree that LoF, often leading to haploinsufficiency, is the
913 pathophysiologic mechanism of mild and self-limited BFNS (267, 268). DEE mutations can induce
914 more severe LoF consistent with a dominant negative effect. A single report showed that the
915 recurrent p.A294V K_V7.2 pore mutation does not modify the properties of the current, but induces
916 LoF altering the subcellular localization of K_V channels (280). DEE can also be caused by GoF, in
917 which case, disease severity correlates with that of functional alteration (267). The first transgenic
918 mouse model expressing a *KCNQ2* dominant-negative mutant (149), showed spontaneous focal
919 and generalized tonic-clonic seizures, impaired hippocampus-related memory and pronounced
920 hyperactivity, but also cell loss in the hippocampus, which is not observed in patients. The first
921 knock-in model of *KCNQ2*-DEE (281) carrying the p.T274M mutation displayed an overall 70–80%
922 reduction of the M-current (282). *KCNQ2*$^{T274M/+}$ mice show spontaneous generalized seizures and
923 impairment of spatial learning and memory, and do not show major structural brain defects or
924 neuronal mortality, which is consistent with the clinical features observed in patients. The
925 selective deletion of *KCNQ2* and *KCNQ3* in parvalbumin-positive interneurons increased their

926 firing, leading to homeostatic potentiation of excitatory synaptic activity. Consistently this model
927 shows reduced latency for picrotoxin-induced seizures (283).

928 **2.2. Delayed rectifier K^+ channels (K_V1.1/*KCNA1*; K_V1.2/*KCNA2*; K_V2.1/*KCNB1*; K_V8.2/*KCNV2*)**

929 K_V1.1 (*KCNA1*) and K_V1.2 (*KCNA2*). *KCNA2* encodes the K_V1.2 shaker-type voltage-gated K^+ channel
930 subunit, which is highly expressed in both excitatory and inhibitory neurons, particularly in the
931 axon (284–286). The K_V1.2 channel generates the delayed rectifier K^+ current, an important
932 component of the repolarizing currents during APs. K_V1.2 channels have high homology to K_V1.1
933 channels but require stronger depolarization to activate. K_V1.1 and K_V1.2 subunits can produce
934 heteromeric potassium channels with intermediate properties between the respective homomers
935 (287). K_V1.1 and K_V1.2 subunits are associated with cell adhesion molecules (CAMs), including
936 LGI1, which contributes to their subcellular localization (288). *KCNA2*-DEE clinical features have
937 been correlated with effects of mutations studied *in vitro* and can result in strong GoF, mixed GoF
938 and LoF, and LoF with negative dominance (289, 290). Patients with strong GoF variants have
939 onset between five and 15 months of age with intellectual disability, ataxia, and cerebellar
940 atrophy, often with generalized seizures. Patients with variants showing mixed GoF and LoF effects
941 have onset from the neonatal period to six months of age with refractory generalized and focal
942 seizures and developmental delay. Patients with dominant negative LoF variants can have less
943 severe features. The LoF mechanism is consistent with the classical role of K^+ channels and the
944 phenotype of K_V1.2 mutant mice. Homozygous knock-out mice show severe seizures and early
945 mortality, and heterozygous knock-out mice are more sensitive to convulsants, although they do
946 not show spontaneous seizures (291). Both heterozygous and homozygous "*Pingu*" mice, which
947 carry a chemically induced LoF missense mutation, show motor abnormalities (292). Since K_V1.2
948 channels are expressed in both excitatory and inhibitory neurons, the cellular mechanism of
949 *KCNA2* mutations is not completely clear. Their repolarizing role can help both sustaining high

950 firing rates and setting the resting membrane potential. Recordings from LoF mouse models have
951 shown contrasting modifications of neuronal firing, with hypoexcitability of glycinergic neurons in
952 *Kcna2* knock-out (291) and hyperexcitability of cerebellar basket cells in *Pingu* mice (292).
953 Although the detailed pathological mechanism is still elusive, the identification of GoF or LoF
954 effects can provide important information for orienting the therapy in a precision medicine
955 approach. In fact, it has been recently shown that the K^+ channel blocker 4-aminopyridine
956 antagonized GoF defects caused by variants in *KCNA2 in vitro* and was effective in reducing
957 symptoms in patients carrying GoF *KCNA2* mutations (293). *KCNA1* LoF mutations have also been
958 identified in some DEE patients (294, 295) extending the pathological spectrum of this gene to
959 severe epilepsy.
960 $K_V2.1$ (*KCNB1*) and $K_V8.2$ (*KCNV2*). *KCNB1* encodes the $K_V2.1$ pore-forming and voltage-sensing α-
961 subunit, which contributes to generate the delayed-rectifier K^+ current (268). It is expressed in
962 both excitatory and inhibitory neurons of the mammalian brain, and is localized to the soma,
963 proximal dendrites, and axon initial segments (296). $K_V2.1$ is important for sensing and
964 homeostatically regulating excitability, because its activity can be inhibited by phosphorylation;
965 increased neuronal activity induces dephosphorylation, which results in increased delayed rectifier
966 current leading to reduced neuronal excitability (297, 298). Mutations in *KCNB1* have been
967 recently reported in patients with early-onset DEE (299–301). *KCNB1*-related DEEs encompass a
968 wide spectrum of neurodevelopmental disorders with different types of epileptic seizures,
969 predominant language difficulties and behavioral impairment. Most variants occur *de novo* and
970 mainly consist of missense variants located on the voltage sensor and the pore domain. Truncating
971 variants in the C-terminal domain are associated with a less-severe epileptic phenotype, although
972 cognitive/behavioral impairment is still severe. Functional studies in transfected cells have shown
973 a variety of defects, including loss of ion selectivity, reduced conductance, and dominant negative

974 effects, as well as milder effects on gating properties (299, 302, 303), which induce reduced
975 function and increased neuronal excitability. *Kcnb1*$^{-/-}$ mice show hyperexcitable hippocampal
976 circuits but do not show spontaneous seizures, although they exhibit increased seizure
977 susceptibility (298). The voltage-gated K^+ channel subunit K_V8.2, encoded by the *KCNV2* gene, is a
978 silent subunit when expressed as a homotetramer, but it increases the K_V2 current when co-
979 assembled as a hetero-tetramer with other K_V2 channels, for example in hippocampal pyramidal
980 neurons, in which it co-localize with Kv2.1 and contributes to the generation of the delayed-
981 rectifier K^+ current (304, 305). *De novo* LoF variants in the *KCNV2* gene, encoding the voltage-
982 gated K^+ channel subunit K_V8.2, have been identified in few DEE patients and contribute to
983 epilepsy susceptibility in mice (305).

984 **2.3. Fast K^+ channels**

985 K_V3.1 (*KCNC1*) and K_V4.1-4.3 (*KCND*). *KCNC1* encodes the K_V3.1 channel, which is preferentially
986 expressed in neurons that generate high frequency firing, including parvalbumin-containing
987 GABAergic interneurons in the cerebral cortex, hippocampus and amygdala, auditory brainstem
988 neurons, cerebellar granule cells, and neurons of the reticular nucleus of the thalamus (306). In
989 fast spiking GABAergic neurons, K_V3.1 channels are localized to the proximal dendrites, somata,
990 axon hillock, and synaptic terminals, but are not found in distal dendrites (306, 307). Specific
991 functional properties that distinguish K_V3.1 channels from other K_V channels are the very fast
992 kinetics of activation and deactivation, and the voltage dependence of activation shifted towards
993 depolarized potentials (306). These properties are optimized for promoting the generation of high
994 firing rates, up to hundreds of Hz (308). Mutations in *KCNC1* have been identified in early onset
995 DEE and in patients with intellectual disability without seizures (309, 310). Mutants have been
996 functionally characterized in the *Xenopus laevis* oocytes. All characterized mutations resulted in
997 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 *Kcnc1*$^{-/-}$ mice have a subtle phenotype (312) and there are no mouse models engineered to carry human *KCNC1* mutations.

KCND1-3 encode the K_V4.1-3 alpha 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 *de novo* heterozygous missense variant p.V404M of *KCND2* has been associated with DEE with onset at 2 months (314). Functional analysis in *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 K_V10.1 (EAG1) and K_V10.2 (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 K_V10.1 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 *KCHN1* 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. *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^{2+} activated K^+ channels

KCNMA1 encodes the pore-forming α-subunit of the large-conductance $K_{Ca}1.1$ Ca^{2+} activated K^+ channel ("Big K^+", BK), which is activated by depolarizations and intracellular Ca^{2+} (323). $K_{Ca}1.1$ is widely distributed in excitable and non-excitable 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 *de novo* missense mutations in *KCNMA1* are associated with a wide phenotypic spectrum primarily defined by brain and muscle dysfunction (324–326). *KCNMA1*-linked DEE is characterized by a heterogeneous combination of epilepsy, dyskinesia, and intellectual disability. Functional analysis in cell lines transfected with *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. *Kcnma1*$^{-/-}$ mice show ataxia, tremor, impaired coordination and spatial learning (327, 328). Pharmacological inhibition of $K_{Ca}1.1$ channels induce tremor and ataxia in animals (329), but they might be used for treatment of GoF mutations.

2.6. Na^+-activated K^+ channels

KCNT1 encodes the $K_{Na}1.1$ subunit, which has a classical six transmembrane segments structure and forms a tetrameric Na^+-activated K^+ channel (also called Slack, KCa4.1 or Slo2.2). $K_{Na}1.1$ is activated by both intracellular Na^+ 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 $K_{Na}1.2$ (encoded by the gene *KCNT2*), but the two subunits can co-localize and form heteromeric channels (330, 331). K_{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 Na^+ influx through Na_V channels (332).

Mutations in *KCNT1* cause different phenotypes, including DEEs. *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. *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 $K_{Na}1.1$ current amplitude (333, 336), while one heterozygous LoF variant (p.F932I) from a patient with severe generalized seizures and delayed myelination causes impaired $K_{Na}1.1$ trafficking (337). Knock-in mice of the p.Y796H GoF *KCNT1* mutation show early-onset seizures (338). Although $K_{Na}1.1$ increases K_{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. Ca^{2+} CHANNELS

Voltage-gated Ca^{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^{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; *CACNA1S/*$Ca_V1.1$, *CACNA1C/*$Ca_V1.2$, *CACNA1/*$Ca_V1.3$, and *CACNA1F/*$Ca_V1.4$), the high/moderate-voltage activated dihydropyridine-insensitive channels (P/Q-, N- and R- type; *CACNA1A*/$Ca_V2.1$, *CACNA1B*/$Ca_V2.2$ and *CACNA1E*/$Ca_V2.3$), and the low-voltage-activated (T-type; *CACNA1G*/$Ca_V3.1$, *CACNA1H*/$Ca_V3.2$ and *CACNA1I*/$Ca_V3.3$) 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 *CACNA1A*/$Ca_V2.1$ 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).

3.1 T-type channels

T-type channels *(CACNA1G/H/I, $Ca_V3.1$-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*/$Ca_V3.1$ and *CACNA1H*/$Ca_V3.2$ causing in general mild GoF have been identified as risk factors for idiopathic generalized epilepsy (349). *De novo* heterozygous missense variants in *CACNA1G*/$Ca_V3.1$ and *CACNA1I*//$Ca_V3.1$ 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 Ca^{2+} current at resting membrane potential for *CACNA1I*//$Ca_V3.1$ variants), or mixed GoF and LoF. Evidence for the involvement of *CACNA1H*/$Ca_V3.2$ in DEE is more limited. Gene targeted animal models of T-type channels DEE mutations are not available yet.

3.2. P/Q-type Ca^{2+} channels

CACNA1A encodes the $Ca_v2.1$ Ca^{2+} channel, which generates the high voltage-activated P/Q-type Ca^{2+} 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 Ca^{2+} channels

CACNA1B encodes the $Ca_v2.2$ N-type Ca^{2+} channel, which generates the high voltage-activated Ca^{2+} 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 $Ca_v2.2$ channels are replaced by CaV2.1 channels in most mature synapses (353). In particular, $Ca_v2.2$ is implicated in asynchronous synaptic release, which occurs up to tens of seconds after the AP (353). $Ca_v2.2$ 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 $Ca_v2.2$ 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 $Ca_V2.3$ 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 ($Na_VI2.1$) 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 Ca^{2+} 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^+/Ca^{2+} 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 H_{abc} 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^{2+} channels and upon Ca^{2+} binding trigger membrane deformation and fusion (413, 414). Five heterozygous mutations were reported in Synaptotagmin-1 located at the Ca^{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^+ channel antagonists (400). Presynaptic voltage-gated Ca^{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^{2+} sensing machinery (synaptotagmin 1/2, SNAP-25 and VAMP2) boosting the Ca^{2+} sensitivity of NT release and suggesting a function in the Ca^{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^{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,

426). 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, *GRIN1*, *GRIN2A* and *GRIN2B* are the target of most variants identified in patients with neurodevelopmental disorders. Epilepsy in patients carrying *GRIN* mutations has onset from birth to few years of early childhood. Mutations can be either inherited or *de novo* and generate a spectrum of phenotypes ranging from mild intellectual disability to severe DEE (458). The *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). *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 *GRIN2B* spectrum, characterized by various GoF and LoF mechanisms, is similar to the *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 *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 *GRIN* genes (458). Mice carrying homozygous null mutations for *Grin1* and *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). *Grin2a*$^{+S/644G}$ *and Grin2a*$^{+/N615K}$ mice show perinatal lethality in homozygosis, increased seizure propensity, behavioral and cognitive deficits in heterozygosis. *Grin2b*$^{+/C456Y}$ 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^{2+} 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*$^{-/-}$ 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, GABRR1–3, 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 *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).

SLC12A5 recessive *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 *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 *CLCN* genes leads to severe neurological disorders, in particular LoF mutations in *CLCN4* cause a spectrum of phenotypes including severe DEE with drug-resistant seizures, cognitive and behavioral disorders (494, 495).

SLC1A2 AND *SLC6A1*. 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 *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. *Slc1a2* knockout mice show a severe epilepsy phenotype only in homozygosity (497).

The *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 *SLC6A1* DEE mutations induce LoF of GAT1 possibly associated with negative dominance and reduced GABA reuptake (481). *Slc6a1*$^{-/-}$ mice recapitulate some features of the human phenotypes, including motor and cognitive impairment, whereas heterozygous *Slc6a1*$^{+/-}$ mice do not show an overt phenotype (498). Overall, the mechanism may be similar to that of *GABRD* mutations, which increase the extrasynaptic $GABA_A$ current (480).

2.3 Other postsynaptic proteins

Other mutations in genes encoding for receptor-associated proteins, such as the GTPase 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).

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 *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 (**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.

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 *Dmxl2*-silenced mouse hippocampal neurons that exhibit impaired neurite elongation and synaptic loss (431). *Dmxl2*$^{-/-}$ mice are embryonic lethal (533) while heterozygous *Dmxl2* mice show brain malformations, uncovering a penetrant role of *Dmxl2* 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 throughput 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 *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 garget 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 $Na_v1.1$ 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 *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 *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).

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 anatomoclinical 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.

ACKNOWLEDGMENTS AND GRANTS

R. Guerrini acknowledges grant support by the Tuscany Region Call for Health 2018 (Project DECODE-EE) and by Fondazione Cassa di Risparmio di Firenze (Project BRAIN). A.S. Galanopoulou acknowledges grant support by NIH U54 NS100064, R01 NS091170, American Epilepsy Society

seed grant, the Heffer Family and the Segal Family Foundations, and the Abbe Goldstein/Joshua Lurie and Laurie Marsh/Dan Levitz families. F. Benfenati acknowledges grant support by Era-Net Neuron 2017 Snaropathies, IRCCS Ospedale Policlinico San Martino (Ricerca Corrente and "5x1000"), the EU Joint Programme on Neurodegenerative Disease Research 2020 Project "Neurophage", and the Italian Ministry of University and Research (PRIN 2015-H4K2CR and 2017-A9MK4R). The support of Telethon-Italy (Grant GGP19120 to FB) is also acknowledged. M. Mantegazza acknowledges grant support by the Laboratory of Excellence "Ion Channel Science and Therapeutics" (LabEx ICST, ANR-11-LABX-0015-01, France) and the IDEX UCA-Jedi (University Côte d'Azur ANR-15-IDEX-01, France). Graphical Abstract was created with BioRender.com.

DISCLOSURES

AS Galanopoulou is the Editor-in-Chief of *Epilepsia Open* and receives royalties from Elsevier, Medlink, and Morgan and Claypool for publications. The other authors declare no conflicts of interest, financial or otherwise.

REFERENCES

1. Stafstrom CE, Carmant L. Seizures and Epilepsy: An Overview for Neuroscientists. **Cold Spring Harb Perspect Med** 5: a022426--a022426, 2015. doi: 10.1101/cshperspect.a022426.
2. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. **Epilepsia** 58: 512–521, 2017. doi: 10.1111/epi.13709.
3. Cutri-French C, Armstrong D, Saby J, Gorman C, Lane J, Fu C, et al. Comparison of Core Features in Four Developmental Encephalopathies in the Rett Natural History Study. **Ann Neurol** 88: 396–406, 2020. doi: 10.1002/ana.25797.
4. McTague A, Howell KB, Cross JH, Kurian MA, Scheffer IE. The genetic landscape of the epileptic encephalopathies of infancy and childhood. **Lancet Neurol** 15: 304–316, 2016. doi:

10.1016/S1474-4422(15)00250-1.

5. Riney K, Bogacz A, Somerville E, Hirsch E, Nabbout R, Scheffer IE, et al. ILAE Classification and Definition of Epilepsy Syndromes with Onset at a Variable Age: Position Statement by the ILAE Task Force on Nosology and Definitions. [Online]. Edited by ILAE 2021: 2021. http://files/1501/Riney_VariableAges_7Apr21.pdf.

6. Specchio N, Wirrel EC, Scheffer IE, Nabbout R, Riney K, Samia P, et al. ILAE Classification and Definition of Epilepsy Syndromes with Onset in Childhood: Position Paper by the ILAE Task Force on Nosology and Definitions. [Online]. Edited by ILAE 2021: 2021. http://files/1503/Specchio.

7. Zuberi SM, Wirrel EC, Yozawitz E, Wilmshurst JM, Specchio N, Riney K, et al. ILAE Classification & Definition of Epilepsy Syndromes in the Neonate and Infant: Position Statement by the ILAE Task Force on Nosology and Definitions [Online]. Edited by ILAE 2021: 2021. http://files/1505/Zuberi.

8. Bayat A, Bayat M, Rubboli G, Møller RS. Epilepsy Syndromes in the First Year of Life and Usefulness of Genetic Testing for Precision Therapy. **Genes** 12: 1051, 2021. doi: 10.3390/genes12071051.

9. Consortium E, Project EP, Allen AS, Berkovic SF, Cossette P, Delanty N, et al. De novo mutations in epileptic encephalopathies. **Nature** 501: 217–221, 2013. doi: 10.1038/nature12439.

10. Epilepsy Phenome/Genome Project, Epi4K Consortium. Copy number variant analysis from exome data in 349 patients with epileptic encephalopathy. **Ann Neurol** 78: 323–328, 2015. doi: 10.1002/ana.24457.

11. Epi4K Consortium, Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, et al. De novo mutations in synaptic transmission genes including DNM1 cause

epileptic encephalopathies. **Am J Hum Genet** 95: 360–370, 2014. doi: 10.1016/j.ajhg.2014.08.013.

12. Paciorkowski AR, Thio LL, Dobyns WB. Genetic and biologic classification of infantile spasms. **Pediatr Neurol** 45: 355–367, 2011. doi: 10.1016/j.pediatrneurol.2011.08.010.

13. Wirrell EC, Shellhaas RA, Joshi C, Keator C, Kumar S, Mitchell WG, et al. How should children with West syndrome be efficiently and accurately investigated? Results from the National Infantile Spasms Consortium. **Epilepsia** 56: 617–625, 2015. doi: 10.1111/epi.12951.

14. Asadi-Pooya AA. Lennox-Gastaut syndrome: a comprehensive review. **Neurol Sci** 39: 403–414, 2018. doi: 10.1007/s10072-017-3188-y.

15. Ben-Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. **Lancet Neurol** 5: 1055–1063, 2006. doi: 10.1016/S1474-4422(06)70626-3.

16. Hamdan FF, Myers CT, Cossette P, Lemay P, Spiegelman D, Laporte AD, et al. High Rate of Recurrent De Novo Mutations in Developmental and Epileptic Encephalopathies. **Am J Hum Genet** 101: 664–685, 2017. doi: 10.1016/j.ajhg.2017.09.008.

17. Mefford HC, Yendle SC, Hsu C, Cook J, Geraghty E, McMahon JM, et al. Rare copy number variants are an important cause of epileptic encephalopathies. **Ann Neurol** 70: 974–985, 2011. doi: 10.1002/ana.22645.

18. Ma Y, Chen C, Wang Y, Wu L, He F, Chen C, et al. Analysis copy number variation of Chinese children in early-onset epileptic encephalopathies with unknown cause: CNVs analysis in EOEEs. **Clin Genet** 90: 428–436, 2016. doi: 10.1111/cge.12768.

19. Happ HC, Carvill GL. A 2020 View on the Genetics of Developmental and Epileptic Encephalopathies. **Epilepsy Curr** 20: 90–96, 2020. doi: 10.1177/1535759720906118.

20. Myers CT, Hollingsworth G, Muir AM, Schneider AL, Thuesmunn Z, Knupp A, et al. Parental Mosaicism in “De Novo” Epileptic Encephalopathies. **N Engl J Med** 378: 1646–1648, 2018.

1976 doi: 10.1056/NEJMc1714579.

1977 21. de Lange IM, Koudijs MJ, van 't Slot R, Sonsma ACM, Mulder F, Carbo EC, et al. Assessment
1978 of parental mosaicism in *SCN1A* -related epilepsy by single-molecule molecular inversion
1979 probes and next-generation sequencing. **J Med Genet** 56: 75–80, 2019. doi:
1980 10.1136/jmedgenet-2018-105672.

1981 22. Helbig KL, Farwell Hagman KD, Shinde DN, Mroske C, Powis Z, Li S, et al. Diagnostic exome
1982 sequencing provides a molecular diagnosis for a significant proportion of patients with
1983 epilepsy. **Genet Med** 18: 898–905, 2016. doi: 10.1038/gim.2015.186.

1984 23. Papuc SM, Abela L, Steindl K, Begemann A, Simmons TL, Schmitt B, et al. The role of
1985 recessive inheritance in early-onset epileptic encephalopathies: a combined whole-exome
1986 sequencing and copy number study. **Eur J Hum Genet** 27: 408–421, 2019. doi:
1987 10.1038/s41431-018-0299-8.

1988 24. Carvill GL, Engel KL, Ramamurthy A, Cochran JN, Roovers J, Stamberger H, et al. Aberrant
1989 Inclusion of a Poison Exon Causes Dravet Syndrome and Related SCN1A-Associated Genetic
1990 Epilepsies. **Am J Hum Genet** 103: 1022–1029, 2018. doi: 10.1016/j.ajhg.2018.10.023.

1991 25. Zhang X, Chen MH, Wu X, Kodani A, Fan J, Doan R, et al. Cell-Type-Specific Alternative
1992 Splicing Governs Cell Fate in the Developing Cerebral Cortex. **Cell** 166: 1147--1162.e15,
1993 2016. doi: 10.1016/j.cell.2016.07.025.

1994 26. D'Gama AM, Walsh CA. Somatic mosaicism and neurodevelopmental disease. **Nat Neurosci**
1995 21: 1504–1514, 2018. doi: 10.1038/s41593-018-0257-3.

1996 27. Winawer MR, Griffin NG, Samanamud J, Baugh EH, Rathakrishnan D, Ramalingam S, et al.
1997 Somatic SLC35A2 variants in the brain are associated with intractable neocortical epilepsy.
1998 **Ann Neurol** 83: 1133–1146, 2018. doi: 10.1002/ana.25243.

1999 28. Sim NS, Seo Y, Lim JS, Kim WK, Son H, Kim HD, et al. Brain somatic mutations in *SLC35A2*

cause intractable epilepsy with aberrant N-glycosylation. **Neurol Genet** 4: e294, 2018. doi: 10.1212/NXG.0000000000000294.

29. Baldassari S, Ribierre T, Marsan E, Adle-Biassette H, Ferrand-Sorbets S, Bulteau C, et al. Dissecting the genetic basis of focal cortical dysplasia: a large cohort study. **Acta Neuropathol** 138: 885–900, 2019. doi: 10.1007/s00401-019-02061-5.

30. Miller KE, Koboldt DC, Schieffer KM, Bedrosian TA, Crist E, Sheline A, et al. Somatic *SLC35A2* mosaicism correlates with clinical findings in epilepsy brain tissue. **Neurol Genet** 6: e460, 2020. doi: 10.1212/NXG.0000000000000460.

31. Bonduelle T, Hartlieb T, Baldassari S, Sim NS, Kim SH, Kang H-C, et al. Frequent SLC35A2 brain mosaicism in mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE). **Acta Neuropathol Commun** 9: 3, 2021. doi: 10.1186/s40478-020-01085-3.

32. Nakayama T, Ishii A, Yoshida T, Nasu H, Shimojima K, Yamamoto T, et al. Somatic mosaic deletions involving *SCN1A* cause Dravet syndrome. **Am J Med Genet Part A** 176: 657–662, 2018. doi: 10.1002/ajmg.a.38596.

33. Stosser MB, Lindy AS, Butler E, Retterer K, Piccirillo-Stosser CM, Richard G, et al. High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. **Genet Med** 20: 403–410, 2018. doi: 10.1038/gim.2017.114.

34. Ye Z, Chatterton Z, Pflueger J, Damiano JA, McQuillan L, Harvey AS, et al. Cerebrospinal fluid liquid biopsy for detecting somatic mosaicism in brain. **Brain Commun** 3: fcaa235, 2021. doi: 10.1093/braincomms/fcaa235.

35. Kim S, Baldassari S, Sim NS, Chipaux M, Dorfmüller G, Kim DS, et al. Detection of Brain Somatic Mutations in Cerebrospinal Fluid from Refractory Epilepsy Patients. **Ann Neurol** 89: 1248–1252, 2021. doi: 10.1002/ana.26080.

2024 36. Eichers E, Lewis RA, Katsanis N, Lupski J. Triallelic inheritance: a bridge between Mendelian
2025 and multifactorial traits. **Ann Med** 36: 262–272, 2004. doi: 10.1080/07853890410026214.
2026 37. Glasscock E, Qian J, Yoo JW, Noebels JL. Masking epilepsy by combining two epilepsy genes.
2027 **Nat Neurosci** 10: 1554–1558, 2007. doi: 10.1038/nn1999.
2028 38. Hawkins NA, Martin MS, Frankel WN, Kearney JA, Escayg A. Neuronal voltage-gated ion
2029 channels are genetic modifiers of generalized epilepsy with febrile seizures plus. **Neurobiol**
2030 **Dis** 41: 655–660, 2011. doi: 10.1016/j.nbd.2010.11.016.
2031 39. Klassen T, Davis C, Goldman A, Burgess D, Chen T, Wheeler D, et al. Exome Sequencing of
2032 Ion Channel Genes Reveals Complex Profiles Confounding Personal Risk Assessment in
2033 Epilepsy. **Cell** 145: 1036–1048, 2011. doi: 10.1016/j.cell.2011.05.025.
2034 40. Hasan S, Balobaid A, Grottesi A, Dabbagh O, Cenciarini M, Rawashdeh R, et al. Lethal digenic
2035 mutations in the K^+ channels Kir4.1 (*KCNJ10*) and SLACK (*KCNT1*) associated with severe-
2036 disabling seizures and neurodevelopmental delay. **J Neurophysiol** 118: 2402–2411, 2017.
2037 doi: 10.1152/jn.00284.2017.
2038 41. Calhoun JD, Hawkins NA, Zachwieja NJ, Kearney JA. *Cacna1g* is a genetic modifier of
2039 epilepsy in a mouse model of Dravet syndrome. **Epilepsia** 58: e111--e115, 2017. doi:
2040 10.1111/epi.13811.
2041 42. Fernández-Marmiesse A, Roca I, Díaz-Flores F, Cantarín V, Pérez-Poyato M, Fontalba A, et al.
2042 Rare Variants in 48 Genes Account for 42% of Cases of Epilepsy With or Without
2043 Neurodevelopmental Delay in 246 Pediatric Patients. **Front Neurosci** 13: 1135, 2019. doi:
2044 10.3389/fnins.2019.01135.
2045 43. Pelorosso C, Watrin F, Conti V, Buhler E, Gelot A, Yang X, et al. Somatic double-hit in MTOR
2046 and RPS6 in hemimegalencephaly with intractable epilepsy. **Hum Mol Genet** 28: 3755–
2047 3765, 2019. doi: 10.1093/hmg/ddz194.

2048 44. Horsthemke B. Epimutations in human disease. **Curr Top Microbiol Immunol** 310: 45–59,
2049 2006. doi: 10.1007/3-540-31181-5_4.

2050 45. Henshall DC, Kobow K. Epigenetics and Epilepsy. **Cold Spring Harb Perspect Med** 5: a022731,
2051 2015. doi: 10.1101/cshperspect.a022731.

2052 46. Urdinguio RG, Sanchez-Mut J V, Esteller M. Epigenetic mechanisms in neurological diseases:
2053 genes, syndromes, and therapies. **Lancet Neurol** 8: 1056–1072, 2009. doi: 10.1016/S1474-
2054 4422(09)70262-5.

2055 47. Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai L-H. Recovery of learning and memory is
2056 associated with chromatin remodelling. **Nature** 447: 178–182, 2007. doi:
2057 10.1038/nature05772.

2058 48. Levenson JM, Sweatt JD. Epigenetic mechanisms in memory formation. **Nat Rev Neurosci** 6:
2059 108–118, 2005. doi: 10.1038/nrn1604.

2060 49. Levenson JM, Roth TL, Lubin FD, Miller CA, Huang I-C, Desai P, et al. Evidence That DNA
2061 (Cytosine-5) Methyltransferase Regulates Synaptic Plasticity in the Hippocampus. **J Biol**
2062 **Chem** 281: 15763–15773, 2006. doi: 10.1074/jbc.M511767200.

2063 50. Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T. Mouse and ratBDNF gene structure and
2064 expression revisited. **J Neurosci Res** 85: 525–535, 2007. doi: 10.1002/jnr.21139.

2065 51. Binder DK. The Role of BDNF in Epilepsy and Other Diseases of the Mature Nervous System.
2066 In: **Recent Advances in Epilepsy Research**, edited by Binder DK, Scharfman HE. Springer US,
2067 p. 34–56. http://link.springer.com/10.1007/978-1-4757-6376-8_3.

2068 52. Nelson ED, Kavalali ET, Monteggia LM. Activity-Dependent Suppression of Miniature
2069 Neurotransmission through the Regulation of DNA Methylation. **J Neurosci** 28: 395–406,
2070 2008. doi: 10.1523/JNEUROSCI.3796-07.2008.

2071 53. Salar S, Moshé SL, Galanopoulou AS. Metabolic etiologies in West syndrome. **Epilepsia**

Open 3: 134–166, 2018. doi: 10.1002/epi4.12102.

54. Galanopoulou AS, Gorter JA, Cepeda C. Finding a better drug for epilepsy: the mTOR pathway as an antiepileptogenic target. **Epilepsia** 53: 1119–1130, 2012. doi: 10.1111/j.1528-1167.2012.03506.x.

55. Katsarou A-M, Moshé SL, Galanopoulou AS. Interneuronopathies and their role in early life epilepsies and neurodevelopmental disorders. **Epilepsia Open** 2: 284–306, 2017. doi: 10.1002/epi4.12062.

56. Lado FA, Rubboli G, Capovilla P, Avanzini G, Moshé SL. Pathophysiology of epileptic encephalopathies. **Epilepsia** 54: 6–13, 2013. doi: 10.1111/epi.12417.

57. Scharfman HE. The neurobiology of epilepsy. **Curr Neurol Neurosci Rep** 7: 348–354, 2007. doi: 10.1007/s11910-007-0053-z.

58. Carvill GL, Regan BM, Yendle SC, O'Roak BJ, Lozovaya N, Bruneau N, et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. **Nat Genet** 45: 1073–1076, 2013. doi: 10.1038/ng.2727.

59. Li D, Yuan H, Ortiz-Gonzalez XR, Marsh ED, Tian L, McCormick EM, et al. GRIN2D Recurrent De Novo Dominant Mutation Causes a Severe Epileptic Encephalopathy Treatable with NMDA Receptor Channel Blockers. **Am J Hum Genet** 99: 802–816, 2016. doi: 10.1016/j.ajhg.2016.07.013.

60. Molinari F, Raas-Rothschild A, Rio M, Fiermonte G, Encha-Razavi F, Palmieri L, et al. Impaired Mitochondrial Glutamate Transport in Autosomal Recessive Neonatal Myoclonic Epilepsy. **Am J Hum Genet** 76: 334–339, 2005. doi: 10.1086/427564.

61. Myers CT, McMahon JM, Schneider AL, Petrovski S, Allen AS, Carvill GL, et al. De Novo Mutations in SLC1A2 and CACNA1A Are Important Causes of Epileptic Encephalopathies. **Am J Hum Genet** 99: 287–298, 2016. doi: 10.1016/j.ajhg.2016.06.003.

62. Lemke JR, Hendrickx R, Geider K, Laube B, Schwake M, Harvey RJ, et al. GRIN2B mutations in West syndrome and intellectual disability with focal epilepsy. **Ann Neurol** 75: 147–154, 2014. doi: 10.1002/ana.24073.

63. Lemke JR, Geider K, Helbig KL, Heyne HO, Schütz H, Hentschel J, et al. Delineating the *GRIN1* phenotypic spectrum: A distinct genetic NMDA receptor encephalopathy. **Neurology** 86: 2171–2178, 2016. doi: 10.1212/WNL.0000000000002740.

64. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. **Nat Neurosci** 9: 1142–1149, 2006. doi: 10.1038/nn1754.

65. Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I, et al. Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. **J Neurosci** 27: 5903–5914, 2007. doi: 10.1523/JNEUROSCI.5270-06.2007.

66. Catterall WA, Kalume F, Oakley JC. $Na_V1.1$ channels and epilepsy: $Na_V1.1$ channels and epilepsy. **J Physiol** 588: 1849–1859, 2010. doi: 10.1113/jphysiol.2010.187484.

67. Du J, Simmons S, Brunklaus A, Adiconis X, Hession CC, Fu Z, et al. Differential excitatory vs inhibitory SCN expression at single cell level regulates brain sodium channel function in neurodevelopmental disorders. **Eur J Paediatr Neurol** 24: 129–133, 2020. doi: 10.1016/j.ejpn.2019.12.019.

68. Hurni N, Kolodziejczak M, Tomasello U, Badia J, Jacobshagen M, Prados J, et al. Transient Cell-intrinsic Activity Regulates the Migration and Laminar Positioning of Cortical Projection Neurons. **Cereb Cortex** 27: 3052–3063, 2017. doi: 10.1093/cercor/bhx059.

69. Vitali I, Fièvre S, Telley L, Oberst P, Bariselli S, Frangeul L, et al. Progenitor Hyperpolarization Regulates the Sequential Generation of Neuronal Subtypes in the Developing Neocortex.

Cell 174: 1264--1276.e15, 2018. doi: 10.1016/j.cell.2018.06.036.

70. Rubinstein M, Westenbroek RE, Yu FH, Jones CJ, Scheuer T, Catterall WA. Genetic background modulates impaired excitability of inhibitory neurons in a mouse model of Dravet syndrome. **Neurobiol Dis** 73: 106–117, 2015. doi: 10.1016/j.nbd.2014.09.017.

71. Pitkänen A, Lukasiuk K, Dudek FE, Staley KJ. Epileptogenesis. **Cold Spring Harb Perspect Med** 5, 2015. doi: 10.1101/cshperspect.a022822.

72. Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. **Epilepsia** 58: 522–530, 2017. doi: 10.1111/epi.13670.

73. Johnston D, Brown TH. The synaptic nature of the paroxysmal depolarizing shift in hippocampal neurons. **Ann Neurol** 16: S65--S71, 1984. doi: 10.1002/ana.410160711.

74. Traub RD, Michelson-Law H, Bibbig AEJ, Buhl EH, Whittington MA. Gap Junctions, Fast Oscillations and the Initiation of Seizures. In: **Recent Advances in Epilepsy Research**, edited by Binder DK, Scharfman HE. Springer US, p. 110–122. http://link.springer.com/10.1007/978-1-4757-6376-8_9.

75. Mylvaganam S, Ramani M, Krawczyk M, Carlen PL. Roles of gap junctions, connexins, and pannexins in epilepsy. **Front Physiol** 5, 2014. doi: 10.3389/fphys.2014.00172.

76. Wallraff A. The Impact of Astrocytic Gap Junctional Coupling on Potassium Buffering in the Hippocampus. **J Neurosci** 26: 5438–5447, 2006. doi: 10.1523/JNEUROSCI.0037-06.2006.

77. Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. Astroglial Metabolic Networks Sustain Hippocampal Synaptic Transmission. **Science** 322: 1551–1555, 2008. doi: 10.1126/science.1164022.

78. Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P. Synchronization of neuronal activity in

hippocampus by individual GABAergic interneurons. **Nature** 378: 75–78, 1995. doi: 10.1038/378075a0.

79. Pinault D, O'brien TJ. Cellular and network mechanisms of genetically-determined absence seizures. **Thalamus Relat Syst** 3: 181–203, 2005. doi: 10.1017/S1472928807000209.

80. Blumenfeld H. Cellular and network mechanisms of spike-wave seizures. **Epilepsia** 46 Suppl 9: 21–33, 2005. doi: 10.1111/j.1528-1167.2005.00311.x.

81. van Luijtelaar G, Onat FY, Gallagher MJ. Animal models of absence epilepsies: what do they model and do sex and sex hormones matter? **Neurobiol Dis** 72 Pt B: 167–179, 2014. doi: 10.1016/j.nbd.2014.08.014.

82. Kostopoulos GK. Spike-and-wave discharges of absence seizures as a transformation of sleep spindles: the continuing development of a hypothesis. **Clin Neurophysiol** 111 Suppl: S27--38, 2000. doi: 10.1016/s1388-2457(00)00399-0.

83. Rosenow F, Lüders H. Presurgical evaluation of epilepsy. **Brain** 124: 1683–1700, 2001. doi: 10.1093/brain/124.9.1683.

84. Lüders HO, Najm I, Nair D, Widdess-Walsh P, Bingman W. The epileptogenic zone: general principles. **Epileptic Disord** 8 Suppl 2: S1--9, 2006. http://www.ncbi.nlm.nih.gov/pubmed/17012067.

85. Kreindler A, Zuckermann E, Steriade M, Chimion D. Electro-clinical features of convulsions induced by stimulation of brain stem. **J Neurophysiol** 21: 430–436, 1958. doi: 10.1152/jn.1958.21.5.430.

86. Neville BG. The origin of infantile spasms: evidence from a case of hydranencephaly. **Dev Med Child Neurol** 14: 644–647, 1972. doi: 10.1111/j.1469-8749.1972.tb02647.x.

87. Lee CL, Frost JD, Swann JW, Hrachovy RA. A new animal model of infantile spasms with unprovoked persistent seizures. **Epilepsia** 49: 298–307, 2008. doi: 10.1111/j.1528-

1167.2007.01377.x.

88. Scantlebury MH, Galanopoulou AS, Chudomelova L, Raffo E, Betancourth D, Moshé SL. A model of symptomatic infantile spasms syndrome. **Neurobiol Dis** 37: 604–612, 2010. doi: 10.1016/j.nbd.2009.11.011.

89. Burgess R, Wang S, McTague A, Boysen KE, Yang X, Zeng Q, et al. The Genetic Landscape of Epilepsy of Infancy with Migrating Focal Seizures. **Ann Neurol** 86: 821–831, 2019. doi: 10.1002/ana.25619.

90. Galanopoulou AS, Moshé SL. In search of epilepsy biomarkers in the immature brain: goals, challenges and strategies. **Biomark Med** 5: 615–628, 2011. doi: 10.2217/bmm.11.71.

91. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. **Early Hum Dev** 3: 79–83, 1979. doi: 10.1016/0378-3782(79)90022-7.

92. Ojeda SR, Advis JP, Andrews WW. Neuroendocrine control of the onset of puberty in the rat. **Fed Proc** 39: 2365–2371, 1980. http://www.ncbi.nlm.nih.gov/pubmed/6989642.

93. Buyanova IS, Arsalidou M. Cerebral White Matter Myelination and Relations to Age, Gender, and Cognition: A Selective Review. **Front Hum Neurosci** 15: 662031, 2021. doi: 10.3389/fnhum.2021.662031.

94. Clancy B, Darlington RB, Finlay BL. Translating developmental time across mammalian species. **Neuroscience** 105: 7–17, 2001. doi: 10.1016/s0306-4522(01)00171-3.

95. Danglot L, Triller A, Marty S. The development of hippocampal interneurons in rodents. **Hippocampus** 16: 1032–1060, 2006. doi: 10.1002/hipo.20225.

96. Lenroot RK, Giedd JN. Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. **Neurosci Biobehav Rev** 30: 718–729, 2006. doi: 10.1016/j.neubiorev.2006.06.001.

97. Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in

rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. **Prog Neurobiol** 106–107: 1–16, 2013. doi: 10.1016/j.pneurobio.2013.04.001.

98. Silbereis JC, Pochareddy S, Zhu Y, Li M, Sestan N. The Cellular and Molecular Landscapes of the Developing Human Central Nervous System. **Neuron** 89: 248–268, 2016. doi: 10.1016/j.neuron.2015.12.008.

99. Steiner P. Brain Fuel Utilization in the Developing Brain. **Ann Nutr Metab** 75 Suppl 1: 8–18, 2019. doi: 10.1159/000508054.

100. Zeiss CJ. Comparative Milestones in Rodent and Human Postnatal Central Nervous System Development. **Toxicol Pathol** 49: 1368–1373, 2021. doi: 10.1177/01926233211046933.

101. Moshé SL, Albala BJ. Maturational changes in postictal refractoriness and seizure susceptibility in developing rats. **Ann Neurol** 13: 552–557, 1983. doi: 10.1002/ana.410130514.

102. Haas KZ, Sperber EF, Moshé SL. Kindling in developing animals: interactions between ipsilateral foci. **Brain Res Dev Brain Res** 68: 140–143, 1992. doi: 10.1016/0165-3806(92)90257-w.

103. Haas KZ, Sperber EF, Opanashuk LA, Stanton PK, Moshé SL. Resistance of immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. **Hippocampus** 11: 615–625, 2001. doi: 10.1002/hipo.1076.

104. Sperber EF, Stanton PK, Haas K, Ackermann RF, Moshé SL. Developmental differences in the neurobiology of epileptic brain damage. **Epilepsy Res Suppl** 9: 67–81, 1992. http://www.ncbi.nlm.nih.gov/pubmed/1285916.

105. Akman O, Moshé SL, Galanopoulou AS. Sex-specific consequences of early life seizures. **Neurobiol Dis** 72 Pt B: 153–166, 2014. doi: 10.1016/j.nbd.2014.05.021.

106. Chudomel O, Hasson H, Bojar M, Moshé SL, Galanopoulou AS. Age- and sex-related characteristics of tonic GABA currents in the rat substantia nigra pars reticulata. **Neurochem Res** 40: 747–757, 2015. doi: 10.1007/s11064-015-1523-3.

107. Chudomel O, Herman H, Nair K, Moshé SL, Galanopoulou AS. Age- and gender-related differences in GABAA receptor-mediated postsynaptic currents in GABAergic neurons of the substantia nigra reticulata in the rat. **Neuroscience** 163: 155–167, 2009. doi: 10.1016/j.neuroscience.2009.06.025.

108. Galanopoulou AS. Sexually dimorphic expression of KCC2 and GABA function. **Epilepsy Res** 80: 99–113, 2008. doi: 10.1016/j.eplepsyres.2008.04.013.

109. Galanopoulou AS, Moshé SL. The epileptic hypothesis: developmentally related arguments based on animal models. **Epilepsia** 50 Suppl 7: 37–42, 2009. doi: 10.1111/j.1528-1167.2009.02217.x.

110. Giorgi FS, Galanopoulou AS, Moshé SL. Sex dimorphism in seizure-controlling networks. **Neurobiol Dis** 72 Pt B: 144–152, 2014. doi: 10.1016/j.nbd.2014.05.010.

111. Kyrozis A, Chudomel O, Moshé SL, Galanopoulou AS. Sex-dependent maturation of GABAA receptor-mediated synaptic events in rat substantia nigra reticulata. **Neurosci Lett** 398: 1–5, 2006. doi: 10.1016/j.neulet.2005.12.018.

112. Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL. Giant synaptic potentials in immature rat CA3 hippocampal neurones. **J Physiol** 416: 303–325, 1989. doi: 10.1113/jphysiol.1989.sp017762.

113. Brown DA, Scholfield CN. Depolarization of neurones in the isolated olfactory cortex of the guinea-pig by gamma-aminobutyric acid. **Br J Pharmacol** 65: 339–345, 1979. doi: 10.1111/j.1476-5381.1979.tb07835.x.

114. Galvan M, Scholfield CN, Brown DA. Depolarizing actions of gamma-aminobutyric acid

(GABA) on mammalian brain slices. **Br J Pharmacol** 62: 410P, 1978. http://www.ncbi.nlm.nih.gov/pubmed/638340.

115. LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. **Neuron** 15: 1287–1298, 1995. doi: 10.1016/0896-6273(95)90008-x.

116. Luhmann HJ, Prince DA. Postnatal maturation of the GABAergic system in rat neocortex. **J Neurophysiol** 65: 247–263, 1991. doi: 10.1152/jn.1991.65.2.247.

117. Mueller AL, Chesnut RM, Schwartzkroin PA. Actions of GABA in developing rabbit hippocampus: an in vitro study. **Neurosci Lett** 39: 193–198, 1983. doi: 10.1016/0304-3940(83)90076-9.

118. Delpire E, Rauchman MI, Beier DR, Hebert SC, Gullans SR. Molecular cloning and chromosome localization of a putative basolateral Na(+)-K(+)-2Cl- cotransporter from mouse inner medullary collecting duct (mIMCD-3) cells. **J Biol Chem** 269: 25677–25683, 1994. http://www.ncbi.nlm.nih.gov/pubmed/7929272.

119. Payne JA, Forbush B. Alternatively spliced isoforms of the putative renal Na-K-Cl cotransporter are differentially distributed within the rabbit kidney. **Proc Natl Acad Sci U S A** 91: 4544–4548, 1994. doi: 10.1073/pnas.91.10.4544.

120. Xu JC, Lytle C, Zhu TT, Payne JA, Benz E, Forbush B. Molecular cloning and functional expression of the bumetanide-sensitive Na-K-Cl cotransporter. **Proc Natl Acad Sci U S A** 91: 2201–2205, 1994. doi: 10.1073/pnas.91.6.2201.

121. Gillen CM, Brill S, Payne JA, Forbush B. Molecular cloning and functional expression of the K-Cl cotransporter from rabbit, rat, and human. A new member of the cation-chloride cotransporter family. **J Biol Chem** 271: 16237–16244, 1996. doi: 10.1074/jbc.271.27.16237.

122. Payne JA, Stevenson TJ, Donaldson LF. Molecular characterization of a putative K-Cl

cotransporter in rat brain. A neuronal-specific isoform. **J Biol Chem** 271: 16245–16252, 1996. doi: 10.1074/jbc.271.27.16245.

123. Ben-Ari Y. Excitatory actions of gaba during development: the nature of the nurture. **Nat Rev Neurosci** 3: 728–739, 2002. doi: 10.1038/nrn920.

124. Galanopoulou AS. GABA receptors as broadcasters of sexually differentiating signals in the brain. **Epilepsia** 46 Suppl 5: 107–112, 2005. doi: 10.1111/j.1528-1167.2005.01007.x.

125. Galanopoulou AS. GABA(A) receptors in normal development and seizures: friends or foes? **Curr Neuropharmacol** 6: 1–20, 2008. doi: 10.2174/157015908783769653.

126. Galanopoulou AS. Dissociated gender-specific effects of recurrent seizures on GABA signaling in CA1 pyramidal neurons: role of GABA(A) receptors. **J Neurosci Off J Soc Neurosci** 28: 1557–1567, 2008. doi: 10.1523/JNEUROSCI.5180-07.2008.

127. Glykys J, Dzhala VI, Kuchibhotla K V, Feng G, Kuner T, Augustine G, et al. Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. **Neuron** 63: 657–672, 2009. doi: 10.1016/j.neuron.2009.08.022.

128. Galanopoulou AS, Kyrozis A, Claudio OI, Stanton PK, Moshé SL. Sex-specific KCC2 expression and GABA(A) receptor function in rat substantia nigra. **Exp Neurol** 183: 628–637, 2003. doi: 10.1016/s0014-4886(03)00213-9.

129. Roux S, Lohof A, Ben-Ari Y, Poulain B, Bossu J-L. Maturation of GABAergic Transmission in Cerebellar Purkinje Cells Is Sex Dependent and Altered in the Valproate Model of Autism. **Front Cell Neurosci** 12: 232, 2018. doi: 10.3389/fncel.2018.00232.

130. Cancedda L, Fiumelli H, Chen K, Poo M. Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. **J Neurosci Off J Soc Neurosci** 27: 5224–5235, 2007. doi: 10.1523/JNEUROSCI.5169-06.2007.

131. Horn Z, Ringstedt T, Blaesse P, Kaila K, Herlenius E. Premature expression of KCC2 in embryonic mice perturbs neural development by an ion transport-independent mechanism. **Eur J Neurosci** 31: 2142–2155, 2010. doi: 10.1111/j.1460-9568.2010.07258.x.

132. Wang DD, Kriegstein AR. Blocking early GABA depolarization with bumetanide results in permanent alterations in cortical circuits and sensorimotor gating deficits. **Cereb Cortex** 21: 574–587, 2011. doi: 10.1093/cercor/bhq124.

133. Galanopoulou AS, Moshé SL. Does epilepsy cause a reversion to immature function? **Adv Exp Med Biol** 813: 195–209, 2014. doi: 10.1007/978-94-017-8914-1_16.

134. Li H, Khirug S, Cai C, Ludwig A, Blaesse P, Kolikova J, et al. KCC2 interacts with the dendritic cytoskeleton to promote spine development. **Neuron** 56: 1019–1033, 2007. doi: 10.1016/j.neuron.2007.10.039.

135. Mavrovic M, Uvarov P, Delpire E, Vutskits L, Kaila K, Puskarjov M. Loss of non-canonical KCC2 functions promotes developmental apoptosis of cortical projection neurons. **EMBO Rep** 21: e48880, 2020. doi: 10.15252/embr.201948880.

136. Winkelmann A, Semtner M, Meier JC. Chloride transporter KCC2-dependent neuroprotection depends on the N-terminal protein domain. **Cell Death Dis** 6: e1776, 2015. doi: 10.1038/cddis.2015.127.

137. Saito T, Ishii A, Sugai K, Sasaki M, Hirose S. A de novo missense mutation in SLC12A5 found in a compound heterozygote patient with epilepsy of infancy with migrating focal seizures. **Clin Genet** 92: 654–658, 2017. doi: 10.1111/cge.13049.

138. Saitsu H, Watanabe M, Akita T, Ohba C, Sugai K, Ong WP, et al. Impaired neuronal KCC2 function by biallelic SLC12A5 mutations in migrating focal seizures and severe developmental delay. **Sci Rep** 6: 30072, 2016. doi: 10.1038/srep30072.

139. Stödberg T, McTague A, Ruiz AJ, Hirata H, Zhen J, Long P, et al. Mutations in SLC12A5 in

epilepsy of infancy with migrating focal seizures. **Nat Commun** 6: 8038, 2015. doi: 10.1038/ncomms9038.

140. Fukata Y, Adesnik H, Iwanaga T, Bredt DS, Nicoll RA, Fukata M. Epilepsy-related ligand/receptor complex LGI1 and ADAM22 regulate synaptic transmission. **Science** 313: 1792–1795, 2006. doi: 10.1126/science.1129947.

141. Velísková J, Moshé SL. Sexual dimorphism and developmental regulation of substantia nigra function. **Ann Neurol** 50: 596–601, 2001. doi: 10.1002/ana.1248.

142. Velísková J, Moshé SL. Update on the role of substantia nigra pars reticulata in the regulation of seizures. **Epilepsy Curr** 6: 83–87, 2006. doi: 10.1111/j.1535-7511.2006.00106.x.

143. Garant DS, Gale K. Substantia nigra-mediated anticonvulsant actions: role of nigral output pathways. **Exp Neurol** 97: 143–159, 1987. doi: 10.1016/0014-4886(87)90289-5.

144. Iadarola MJ, Gale K. Substantia nigra: site of anticonvulsant activity mediated by gamma-aminobutyric acid. **Science** 218: 1237–1240, 1982. doi: 10.1126/science.7146907.

145. Sperber EF, Wurpel JN, Zhao DY, Moshé SL. Evidence for the involvement of nigral GABAA receptors in seizures of adult rats. **Brain Res** 480: 378–382, 1989. doi: 10.1016/0006-8993(89)90211-4.

146. Wurpel JN, Sperber EF, Moshé SL. Age-dependent differences in the anticonvulsant effects of 2-amino-7-phosphono-heptanoic acid or ketamine infusions into the substantia nigra of rats. **Epilepsia** 33: 439–443, 1992. doi: 10.1111/j.1528-1157.1992.tb01688.x.

147. Ikonomidou C, Bittigau P, Koch C, Genz K, Hoerster F, Felderhoff-Mueser U, et al. Neurotransmitters and apoptosis in the developing brain. **Biochem Pharmacol** 62: 401–405, 2001. doi: 10.1016/s0006-2952(01)00696-7.

148. Giorgi FS, Velísková J, Chudomel O, Kyrozis A, Moshé SL. The role of substantia nigra pars

reticulata in modulating clonic seizures is determined by testosterone levels during the immediate postnatal period. **Neurobiol Dis** 25: 73–79, 2007. doi: 10.1016/j.nbd.2006.08.009.

149. Peters HC, Hu H, Pongs O, Storm JF, Isbrandt D. Conditional transgenic suppression of M channels in mouse brain reveals functions in neuronal excitability, resonance and behavior. **Nat Neurosci** 8: 51–60, 2005. doi: 10.1038/nn1375.

150. Olivetti PR, Maheshwari A, Noebels JL. Neonatal estradiol stimulation prevents epilepsy in Arx model of X-linked infantile spasms syndrome. **Sci Transl Med** 6: 220ra12, 2014. doi: 10.1126/scitranslmed.3007231.

151. Chiu C, Reid CA, Tan HO, Davies PJ, Single FN, Koukoulas I, et al. Developmental impact of a familial GABAA receptor epilepsy mutation. **Ann Neurol** 64: 284–293, 2008. doi: 10.1002/ana.21440.

152. Baumbach HD, Chow KL. Visuocortical epileptiform discharges in rabbits: differential effects on neuronal development in the lateral geniculate nucleus and superior colliculus. **Brain Res** 209: 61–76, 1981. doi: 10.1016/0006-8993(81)91172-0.

153. Shatskikh TN, Raghavendra M, Zhao Q, Cui Z, Holmes GL. Electrical induction of spikes in the hippocampus impairs recognition capacity and spatial memory in rats. **Epilepsy Behav** 9: 549–556, 2006. doi: 10.1016/j.yebeh.2006.08.014.

154. Zhou J-L, Lenck-Santini P-P, Zhao Q, Holmes GL. Effect of interictal spikes on single-cell firing patterns in the hippocampus. **Epilepsia** 48: 720–731, 2007. doi: 10.1111/j.1528-1167.2006.00972.x.

155. Akman O, Briggs SW, Mowrey WB, Moshé SL, Galanopoulou AS. Antiepileptogenic effects of rapamycin in a model of infantile spasms due to structural lesions. **Epilepsia** 62: 1985–1999, 2021. doi: 10.1111/epi.16975.

156. Briggs SW, Mowrey W, Hall CB, Galanopoulou AS. CPP-115, a vigabatrin analogue, decreases spasms in the multiple-hit rat model of infantile spasms. **Epilepsia** 55: 94–102, 2014. doi: 10.1111/epi.12424.

157. Galanopoulou AS, Mowrey WB, Liu W, Li Q, Shandra O, Moshé SL. Preclinical Screening for Treatments for Infantile Spasms in the Multiple Hit Rat Model of Infantile Spasms: An Update. **Neurochem Res** 42: 1949–1961, 2017. doi: 10.1007/s11064-017-2282-0.

158. Jequier Gygax M, Klein BD, White HS, Kim M, Galanopoulou AS. Efficacy and tolerability of the galanin analog NAX 5055 in the multiple-hit rat model of symptomatic infantile spasms. **Epilepsy Res** 108: 98–108, 2014. doi: 10.1016/j.eplepsyres.2013.10.015.

159. Katsarou A-M, Li Q, Liu W, Moshé SL, Galanopoulou AS. Acquired parvalbumin-selective interneuronopathy in the multiple-hit model of infantile spasms: A putative basis for the partial responsiveness to vigabatrin analogs? **Epilepsia Open** 3: 155–164, 2018. doi: 10.1002/epi4.12280.

160. Ono T, Moshé SL, Galanopoulou AS. Carisbamate acutely suppresses spasms in a rat model of symptomatic infantile spasms. **Epilepsia** 52: 1678–1684, 2011. doi: 10.1111/j.1528-1167.2011.03173.x.

161. Raffo E, Coppola A, Ono T, Briggs SW, Galanopoulou AS. A pulse rapamycin therapy for infantile spasms and associated cognitive decline. **Neurobiol Dis** 43: 322–329, 2011. doi: 10.1016/j.nbd.2011.03.021.

162. Marsh E, Fulp C, Gomez E, Nasrallah I, Minarcik J, Sudi J, et al. Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. **Brain** 132: 1563–1576, 2009. doi: 10.1093/brain/awp107.

163. Siehr MS, Massey CA, Noebels JL. Arx expansion mutation perturbs cortical development by augmenting apoptosis without activating innate immunity in a mouse model of X-linked

infantile spasms syndrome. **Dis Model Mech** 13, 2020. doi: 10.1242/dmm.042515.

164. Baram TZ. Pathophysiology of massive infantile spasms: perspective on the putative role of the brain adrenal axis. **Ann Neurol** 33: 231–236, 1993. doi: 10.1002/ana.410330302.

165. Brunson KL, Eghbal-Ahmadi M, Baram TZ. How do the many etiologies of West syndrome lead to excitability and seizures? The corticotropin releasing hormone excess hypothesis. **Brain Dev** 23: 533–538, 2001. doi: 10.1016/s0387-7604(01)00312-6.

166. Baram TZ, Schultz L. Corticotropin-releasing hormone is a rapid and potent convulsant in the infant rat. **Brain Res Dev Brain Res** 61: 97–101, 1991. doi: 10.1016/0165-3806(91)90118-3.

167. Shi X-Y, Yang X-F, Tomonoh Y, Hu L-Y, Ju J, Hirose S, et al. Development of a mouse model of infantile spasms induced by N-methyl-D-aspartate. **Epilepsy Res** 118: 29–33, 2015. doi: 10.1016/j.eplepsyres.2015.09.014.

168. Velísek L, Chachua T, Yum M-S, Poon K-L, Velísková J. Model of cryptogenic infantile spasms after prenatal corticosteroid priming. **Epilepsia** 51 Suppl 3: 145–149, 2010. doi: 10.1111/j.1528-1167.2010.02630.x.

169. Yum M-S, Chachua T, Velíšková J, Velíšek L. Prenatal stress promotes development of spasms in infant rats. **Epilepsia** 53: e46--49, 2012. doi: 10.1111/j.1528-1167.2011.03357.x.

170. Wang Y-J, Zhang Y, Liang X-H, Yang G, Zou L-P. Effects of adrenal dysfunction and high-dose adrenocorticotropic hormone on NMDA-induced spasm seizures in young Wistar rats. **Epilepsy Res** 100: 125–131, 2012. doi: 10.1016/j.eplepsyres.2012.02.001.

171. Dubé CM, Molet J, Singh-Taylor A, Ivy A, Maras PM, Baram TZ. Hyper-excitability and epilepsy generated by chronic early-life stress. **Neurobiol Stress** 2: 10–19, 2015. doi: 10.1016/j.ynstr.2015.03.001.

172. Oyrer J, Maljevic S, Scheffer IE, Berkovic SF, Petrou S, Reid CA. Ion Channels in Genetic

Epilepsy: From Genes and Mechanisms to Disease-Targeted Therapies. **Pharmacol Rev** 70: 142–173, 2018. doi: 10.1124/pr.117.014456.

173. Mantegazza M, Rusconi R, Scalmani P, Avanzini G, Franceschetti S. Epileptogenic ion channel mutations: from bedside to bench and, hopefully, back again. **Epilepsy Res** 92: 1–29, 2010. doi: 10.1016/j.eplepsyres.2010.08.003.

174. Guerrini R, Marini C, Mantegazza M. Genetic epilepsy syndromes without structural brain abnormalities: clinical features and experimental models. **Neurotherapeuthics** 11: 269–285, 2014. doi: 10.1007/s13311-014-0267-0.

175. Gawel K, Langlois M, Martins T, van der Ent W, Tiraboschi E, Jacmin M, et al. Seizing the moment: Zebrafish epilepsy models. **Neurosci Biobehav Rev** 116: 1–20, 2020. doi: 10.1016/j.neubiorev.2020.06.010.

176. Shcheglovitov A, Peterson RT. Screening Platforms for Genetic Epilepsies-Zebrafish, iPSC-Derived Neurons, and Organoids. **Neurotherapeutics** 18: 478-1489, 2021. doi: 10.1007/s13311-021-01115-5.

177. Xie Y, Ng NN, Safrina OS, Ramos CM, Ess KC, Schwartz PH, et al. Comparisons of dual isogenic human iPSC pairs identify functional alterations directly caused by an epilepsy associated SCN1A mutation. **Neurobiol Dis** 134: 104627, 2020. doi: 10.1016/j.nbd.2019.104627.

178. Kearney J. The more, the better: Modeling dravet syndrome with induced pluripotent stem cell-derived neurons. **Epilepsy Curr** 14: 33–34, 2014. doi: 10.5698/1535-7597-14.1.33.

179. Liu Y, Lopez-Santiago LF, Yuan Y, Jones JM, Zhang H, O'Malley HA, et al. Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. **Ann Neurol** 74: 128–139, 2013. doi: 10.1002/ana.23897.

180. McTague A, Rossignoli G, Ferrini A, Barral S, Kurian MA. Genome Editing in iPSC-Based

Neural Systems: From Disease Models to Future Therapeutic Strategies. **Front Genome Ed** 3, 2021. doi: 10.3389/fgeed.2021.630600.

181. Kimura Y, Tanaka Y, Shirasu N, Yasunaga S, Higurashi N, Hirose S. Establishment of human induced pluripotent stem cells derived from skin cells of a patient with Dravet syndrome. **Stem Cell Res** 47: 101857, 2020. doi: 10.1016/j.scr.2020.101857.

182. Tanaka Y, Higurashi N, Shirasu N, Yasunaga S, Moreira KM, Okano H, et al. Establishment of a human induced stem cell line (FUi002-A) from Dravet syndrome patient carrying heterozygous R1525X mutation in SCN1A gene. **Stem Cell Res** 31: 11–15, 2018. doi: 10.1016/j.scr.2018.06.008.

183. Sun Y, Paşca SP, Portmann T, Goold C, Worringer KA, Guan W, et al. A deleterious Nav1.1 mutation selectively impairs telencephalic inhibitory neurons derived from Dravet Syndrome patients. **Elife** 5: 1–26, 2016. doi: 10.7554/eLife.13073.

184. Freel BA, Sheets JN, Francis KR. iPSC modeling of rare pediatric disorders. **J Neurosci Methods** 332: 108533, 2020. doi: 10.1016/j.jneumeth.2019.108533.

185. Guerrini R, Dobyns WB. Malformations of cortical development: clinical features and genetic causes. **Lancet Neurol** 13: 710–726, 2014. doi: 10.1016/S1474-4422(14)70040-7.

186. Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB. A developmental and genetic classification for malformations of cortical development: Update 2012. **Brain** 135: 1348–1369, 2012. doi: 10.1093/brain/aws019.

187. Bienvenu T, Poirier K, Friocourt G, Bahi N, Beaumont D, Fauchereau F, et al. ARX, a novel Prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental retardation. **Hum Mol Genet** 11: 981–991, 2002. doi: 10.1093/hmg/11.8.981.

188. Poirier K, Van Esch H, Friocourt G, Saillour Y, Bahi N, Backer S, et al. Neuroanatomical distribution of ARX in brain and its localisation in GABAergic neurons. **Mol Brain Res** 122:

35–46, 2004. doi: 10.1016/j.molbrainres.2003.11.021.

189. Kato M, Dobyns WB. X-linked lissencephaly with abnormal genitalia as a tangential migration disorder causing intractable epilepsy: proposal for a new term, "interneuronopathy". **J Child Neurol** 20: 392–397, 2005. doi: 10.1177/08830738050200042001.

190. Mirzaa GM, Campbell CD, Solovieff N, Goold CP, Jansen LA, Menon S, et al. Association of *MTOR* Mutations With Developmental Brain Disorders, Including Megalencephaly, Focal Cortical Dysplasia, and Pigmentary Mosaicism. **JAMA Neurol** 73: 836, 2016. doi: 10.1001/jamaneurol.2016.0363.

191. Holmes GL, Stafstrom CE, Group ‡The Tuberous Sclerosis Study. Tuberous Sclerosis Complex and Epilepsy: Recent Developments and Future Challenges. **Epilepsia** 48: 617–630, 2007. doi: 10.1111/j.1528-1167.2007.01035.x.

192. Wong M. Mechanisms of Epileptogenesis in Tuberous Sclerosis Complex and Related Malformations of Cortical Development with Abnormal Glioneuronal Proliferation. **Epilepsia** 49: 8–21, 2008. doi: 10.1111/j.1528-1167.2007.01270.x.

193. Cepeda C, Hurst RS, Flores-Hernández J, Hernández-Echeagaray E, Klapstein GJ, Boylan MK, et al. Morphological and electrophysiological characterization of abnormal cell types in pediatric cortical dysplasia: Abnormal Cells in Human Cortical Dysplasia. **J Neurosci Res** 72: 472–486, 2003. doi: 10.1002/jnr.10604.

194. Williams MR, DeSpenza T, Li M, Gulledge AT, Luikart BW. Hyperactivity of Newborn Pten Knock-out Neurons Results from Increased Excitatory Synaptic Drive. **J Neurosci** 35: 943–959, 2015. doi: 10.1523/JNEUROSCI.3144-14.2015.

195. D'Gama AM, Woodworth MB, Hossain AA, Bizzotto S, Hatem NE, LaCoursiere CM, et al. Somatic Mutations Activating the mTOR Pathway in Dorsal Telencephalic Progenitors Cause

a Continuum of Cortical Dysplasias. **Cell Rep** 21: 3754–3766, 2017. doi: 10.1016/j.celrep.2017.11.106.

196. Avoli M, Bernasconi A, Mattia D, Olivier A, Hwa GG. Epileptiform discharges in the human dysplastic neocortex: in vitro physiology and pharmacology. **Ann Neurol** 46: 816–826, 1999. doi: 10.1002/1531-8249(199912)46:6<816::aid-ana3>3.0.co;2-o.

197. Cepeda C, André VM, Levine MS, Salamon N, Miyata H, Vinters H V, et al. Epileptogenesis in pediatric cortical dysplasia: The dysmature cerebral developmental hypothesis. **Epilepsy Behav** 9: 219–235, 2006. doi: 10.1016/j.yebeh.2006.05.012.

198. Talos DM, Kwiatkowski DJ, Cordero K, Black PM, Jensen FE. Cell-specific alterations of glutamate receptor expression in tuberous sclerosis complex cortical tubers. **Ann Neurol** 63: 454–465, 2008. doi: 10.1002/ana.21342.

199. Mantegazza M, Cestèle S, Catterall WA. Sodium channelopathies of skeletal muscle and brain. **Physiol Rev** 101: 1633–1689, 2021. doi: 10.1152/physrev.00025.2020.

200. Stafstrom CE. Persistent Sodium Current and Its Role in Epilepsy. **Epilepsy Curr** 7: 15–22, 2007. doi: 10.1111/j.1535-7511.2007.00156.x.

201. Yu FH, Catterall WA. The VGL-Chanome: A Protein Superfamily Specialized for Electrical Signaling and Ionic Homeostasis. **Sci STKE** 2004, 2004. doi: 10.1126/stke.2532004re15.

202. Mantegazza M, Curia G, Biagini G, Ragsdale DS, Avoli M. Voltage-gated sodium channels as therapeutic targets in epilepsy and other neurological disorders. **Lancet Neurol** 9: 413–424, 2010. doi: 10.1016/S1474-4422(10)70059-4.

203. Meisler MH, Hill SF, Yu W. Sodium channelopathies in neurodevelopmental disorders. **Nat Rev Neurosci** 22: 152–166, 2021. doi: 10.1038/s41583-020-00418-4.

204. Bouza AA, Isom LL. Voltage-Gated Sodium Channel β Subunits and Their Related Diseases. In: **Voltage-gated Sodium Channels: Structure, Function and Channelopathies**, edited by

Chahine M. Springer International Publishing, p. 423–450.

205. Brunklaus A, Lal D. Sodium channel epilepsies and neurodevelopmental disorders: from disease mechanisms to clinical application. **Dev Med Child Neurol** 62: 784–792, 2020. doi: 10.1111/dmcn.14519.

206. Rochtus AM, Goldstein RD, Holm IA, Brownstein CA, Pérez-Palma E, Haynes R, et al. The role of sodium channels in sudden unexpected death in pediatrics. **Mol Genet Genomic Med** 8, 2020. doi: 10.1002/mgg3.1309.

207. Siekierska A, Isrie M, Liu Y, Scheldeman C, Vanthillo N, Lagae L, et al. Gain-of-function FHF1 mutation causes early-onset epileptic encephalopathy with cerebellar atrophy. **Neurology** 86: 2162–2170, 2016. doi: 10.1212/WNL.0000000000002752.

208. Oda Y, Uchiyama Y, Motomura A, Fujita A, Azuma Y, Harita Y, et al. Entire FGF12 duplication by complex chromosomal rearrangements associated with West syndrome. **J Hum Genet** 64: 1005–1014, 2019. doi: 10.1038/s10038-019-0641-1.

209. Mei D, Cetica V, Marini C, Guerrini R. Dravet syndrome as part of the clinical and genetic spectrum of sodium channel epilepsies and encephalopathies. **Epilepsia** 60, 2019. doi: 10.1111/epi.16054.

210. Scheffer IE, Nabbout R. SCN1A-related phenotypes: Epilepsy and beyond. **Epilepsia** 60, 2019. doi: 10.1111/epi.16386.

211. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De Novo Mutations in the Sodium-Channel Gene SCN1A Cause Severe Myoclonic Epilepsy of Infancy. **Am J Hum Genet** 68: 1327–1332, 2001. doi: 10.1086/320609.

212. Dravet C. Dravet syndrome history: Review. **Dev Med Child Neurol** 53: 1–6, 2011. doi: 10.1111/j.1469-8749.2011.03964.x.

213. Depienne C, Arzimanoglou A, Trouillard O, Fedirko E, Baulac S, Saint-Martin C, et al. Parental

mosaicism can cause recurrent transmission of SCN1A mutations associated with severe myoclonic epilepsy of infancy. **Hum Mutat** 27: 389, 2006. doi: 10.1002/humu.9419.

214. Sadleir LG, Mountier EI, Gill D, Davis S, Joshi C, DeVile C, et al. Not all *SCN1A* epileptic encephalopathies are Dravet syndrome: Early profound Thr226Met phenotype. **Neurology** 89: 1035–1042, 2017. doi: 10.1212/WNL.0000000000004331.

215. Gorman KM, Peters CH, Lynch B, Jones L, Bassett DS, King MD, et al. Persistent sodium currents in SCN1A developmental and degenerative epileptic dyskinetic encephalopathy. **Brain Commun** 3, 2021. doi: 10.1093/braincomms/fcab235.

216. Berecki G, Bryson A, Terhag J, Maljevic S, Gazina E V., Hill SL, et al. SCN1A gain of function in early infantile encephalopathy. **Ann Neurol** 85: 514–525, 2019. doi: 10.1002/ana.25438.

217. Bechi G, Scalmani P, Schiavon E, Rusconi R, Franceschetti S, Mantegazza M. Pure haploinsufficiency for Dravet syndrome NaV1.1 (SCN1A) sodium channel truncating mutations. **Epilepsia** 53: 87–100, 2012. doi: 10.1111/j.1528-1167.2011.03346.x.

218. Mantegazza M, Broccoli V. *SCN 1A* /Na_V 1.1 channelopathies: Mechanisms in expression systems, animal models, and human ıPSC models. **Epilepsia** 60, 2019. doi: 10.1111/epi.14700.

219. Terragni B, Scalmani P, Franceschetti S, Cestèle S, Mantegazza M. Post-translational dysfunctions in channelopathies of the nervous system. **Neuropharmacology** 132: 31–42, 2018. doi: 10.1016/j.neuropharm.2017.05.028.

220. Bechi G, Rusconi R, Cestèle S, Striano P, Franceschetti S, Mantegazza M. Rescuable folding defective NaV1.1 (SCN1A) mutants in epilepsy: Properties, occurrence, and novel rescuing strategy with peptides targeted to the endoplasmic reticulum. **Neurobiol Dis** 75: 100–114, 2015. doi: 10.1016/j.nbd.2014.12.028.

221. Rusconi R, Combi R, CestÃ¨le S, Grioni D, Franceschetti S, DalprÃ L, et al. A rescuable folding

defective Na v 1.1 (SCN1A) sodium channel mutant causes GEFS+: Common mechanism in Na v 1.1 related epilepsies? **Hum Mutat** 30: E747--E760, 2009. doi: 10.1002/humu.21041.

222. Rusconi R, Scalmani P, Cassulini RR, Giunti G, Gambardella A, Franceschetti S, et al. Modulatory Proteins Can Rescue a Trafficking Defective Epileptogenic Nav1.1 Na+ Channel Mutant. **J Neurosci** 27: 11037–11046, 2007. doi: 10.1523/JNEUROSCI.3515-07.2007.

223. Thompson CH, Porter JC, Kahlig KM, Daniels MA, George AL. Nontruncating SCN1A Mutations Associated with Severe Myoclonic Epilepsy of Infancy Impair Cell Surface Expression. **J Biol Chem** 287: 42001–42008, 2012. doi: 10.1074/jbc.M112.421883.

224. Han S, Tai C, Westenbroek RE, Yu FH, Cheah CS, Potter GB, et al. Autistic-like behaviour in Scn1a+/- mice and rescue by enhanced GABA-mediated neurotransmission. **Nature** 489: 385–390, 2012. doi: 10.1038/nature11356.

225. Ito S, Ogiwara I, Yamada K, Miyamoto H, Hensch TK, Osawa M, et al. Mouse with Nav1.1 haploinsufficiency, a model for Dravet syndrome, exhibits lowered sociability and learning impairment. **Neurobiol Dis** 49: 29–40, 2013. doi: 10.1016/j.nbd.2012.08.003.

226. Martin MS, Dutt K, Papale LA, Dubé CM, Dutton SB, De Haan G, et al. Altered function of the SCN1A voltage-gated sodium channel leads to γ-aminobutyric acid-ergic (GABAergic) interneuron abnormalities. **J Biol Chem** 285: 9823–9834, 2010. doi: 10.1074/jbc.M109.078568.

227. Hedrich UBS, Liautard C, Kirschenbaum D, Pofahl M, Lavigne J, Liu Y, et al. Impaired Action Potential Initiation in GABAergic Interneurons Causes Hyperexcitable Networks in an Epileptic Mouse Model Carrying a Human NaV1.1 Mutation. **J Neurosci** 34: 14874–14889, 2014. doi: 10.1523/JNEUROSCI.0721-14.2014.

228. Salgueiro-Pereira AR, Duprat F, Pousinha PA, Loucif A, Douchamps V, Regondi C, et al. A two-hit story: Seizures and genetic mutation interaction sets phenotype severity in SCN1A

epilepsies. **Neurobiol Dis** 125: 31–44, 2019. doi: 10.1016/j.nbd.2019.01.006.

229. Chiron C, Marchand MC, Tran A, Rey E, D'Athis P, Vincent J, et al. Stiripentol in severe myoclonic epilepsy in infancy: a randomised placebo-controlled syndrome-dedicated trial. **Lancet** 356: 1638–1642, 2000. doi: 10.1016/S0140-6736(00)03157-3.

230. Ceulemans B, Schoonjans A, Marchau F, Paelinck BP, Lagae L. Five-year extended follow-up status of 10 patients with Dravet syndrome treated with fenfluramine. **Epilepsia** 57, 2016. doi: 10.1111/epi.13407.

231. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. **N Engl J Med** 376: 2011–2020, 2017. doi: 10.1056/NEJMoa1611618.

232. Colasante G, Lignani G, Brusco S, Di Berardino C, Carpenter J, Giannelli S, et al. dCas9-Based Scn1a Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice. **Mol Ther** 28: 235–253, 2020. doi: 10.1016/j.ymthe.2019.08.018.

233. Han Z, Chen C, Christiansen A, Ji S, Lin Q, Anumonwo C, et al. Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. **Sci Transl Med** 12, 2020. doi: 10.1126/scitranslmed.aaz6100.

234. Jansen NA, Dehghani A, Linssen MML, Breukel C, Tolner EA, Maagdenberg AMJM. First FHM3 mouse model shows spontaneous cortical spreading depolarizations. **Ann Clin Transl Neurol** 7: 132–138, 2020. doi: 10.1002/acn3.50971.

235. Auffenberg E, Hedrich UBS, Barbieri R, Miely D, Groschup B, Wuttke T V, et al. Hyperexcitable interneurons trigger cortical spreading depression in an Scn1a migraine model. **J Clin Invest** 131, 2021. doi: 10.1172/JCI142202.

236. Chever O, Zerimech S, Scalmani P, Lemaire L, Pizzamiglio L, Loucif A, et al. Initiation of migraine-related cortical spreading depolarization by hyperactivity of GABAergic neurons

and NaV1.1 channels. **J Clin Invest** 131, 2021. doi: 10.1172/JCI142203.

237. Heron SE, Crossland KM, Andermann E, Phillips HA, Hall AJ, Bleasel A, et al. Sodium-channel defects in benign familial neonatal-infantile seizures. **Lancet** 360: 851–852, 2002. doi: 10.1016/S0140-6736(02)09968-3.

238. Wolff M, Brunklaus A, Zuberi SM. Phenotypic spectrum and genetics of SCN2A-related disorders, treatment options, and outcomes in epilepsy and beyond. **Epilepsia** 60, 2019. doi: 10.1111/epi.14935.

239. Wolff M, Johannesen KM, Hedrich UBS, Masnada S, Rubboli G, Gardella E, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. **Brain** 140: 1316–1336, 2017. doi: 10.1093/brain/awx054.

240. Sanders SJ, Campbell AJ, Cottrell JR, Moller RS, Wagner FF, Auldridge AL, et al. Progress in Understanding and Treating SCN2A-Mediated Disorders. **Trends Neurosci** 41: 442–456, 2018. doi: 10.1016/j.tins.2018.03.011.

241. Spratt PWE, Ben-Shalom R, Keeshen CM, Burke KJ, Clarkson RL, Sanders SJ, et al. The Autism-Associated Gene Scn2a Contributes to Dendritic Excitability and Synaptic Function in the Prefrontal Cortex. **Neuron** 103: 673--685.e5, 2019. doi: 10.1016/j.neuron.2019.05.037.

242. Ogiwara I, Miyamoto H, Tatsukawa T, Yamagata T, Nakayama T, Atapour N, et al. Nav1.2 haplodeficiency in excitatory neurons causes absence-like seizures in mice. **Commun Biol** 1: 96, 2018. doi: 10.1038/s42003-018-0099-2.

243. Wang H-G, Bavley CC, Li A, Jones RM, Hackett JE, Bayleyen Y, et al. Scn2a severe hypomorphic mutation decreases excitatory synaptic input and causes autism-associated behaviors. **JCI Insight** 6: e150698, 2021. doi: 10.1172/jci.insight.150698.

244. Spratt PWE, Alexander RPD, Ben-Shalom R, Sahagun A, Kyoung H, Keeshen CM, et al. Paradoxical hyperexcitability from NaV1.2 sodium channel loss in neocortical pyramidal

cells. **Cell Rep** 36: 109483, 2021. doi: 10.1016/j.celrep.2021.109483.

245. Zhang J, Chen X, Eaton M, Wu J, Ma Z, Lai S, et al. Severe deficiency of the voltage-gated sodium channel NaV1.2 elevates neuronal excitability in adult mice. **Cell Rep** 36: 109495, 2021. doi: 10.1016/j.celrep.2021.109495.

246. Li M, Jancovski N, Jafar-Nejad P, Burbano LE, Rollo B, Richards K, et al. Antisense oligonucleotide therapy reduces seizures and extends life span in an SCN2A gain-of-function epilepsy model. **J Clin Invest** 131, 2021. doi: 10.1172/JCI152079.

247. Vanoye CG, Gurnett CA, Holland KD, George AL, Kearney JA. Novel SCN3A variants associated with focal epilepsy in children. **Neurobiol Dis** 62: 313–322, 2014. doi: 10.1016/j.nbd.2013.10.015.

248. Zaman T, Helbig I, Božović IB, DeBrosse SD, Bergqvist AC, Wallis K, et al. Mutations in SCN3A cause early infantile epileptic encephalopathy. **Ann Neurol** 83: 703–717, 2018. doi: 10.1002/ana.25188.

249. Smith RS, Kenny CJ, Ganesh V, Jang A, Borges-Monroy R, Partlow JN, et al. Sodium Channel SCN3A (NaV1.3) Regulation of Human Cerebral Cortical Folding and Oral Motor Development. **Neuron** 99: 905--913.e7, 2018. doi: 10.1016/j.neuron.2018.07.052.

250. Zaman T, Helbig KL, Clatot J, Thompson CH, Kang SK, Stouffs K, et al. <scp> *SCN3A* </scp> - Related Neurodevelopmental Disorder: A Spectrum of Epilepsy and Brain Malformation. **Ann Neurol** 88: 348–362, 2020. doi: 10.1002/ana.25809.

251. Lamar T, Vanoye CG, Calhoun J, Wong JC, Dutton SBB, Jorge BS, et al. SCN3A deficiency associated with increased seizure susceptibility. **Neurobiol Dis** 102: 38–48, 2017. doi: 10.1016/j.nbd.2017.02.006.

252. Gardella E, Marini C, Trivisano M, Fitzgerald MP, Alber M, Howell KB, et al. The phenotype of SCN8A developmental and epileptic encephalopathy. **Neurology** 91: e1112--e1124, 2018.

doi: 10.1212/WNL.0000000000006199.

253. Johannesen KM, Gardella E, Encinas AC, Lehesjoki A, Linnankivi T, Petersen MB, et al. The spectrum of intermediate SCN 8A -related epilepsy. **Epilepsia** 60: 830–844, 2019. doi: 10.1111/epi.14705.

254. Johannesen KM, Liu Y, Koko M, Gjerulfsen CE, Sonnenberg L, Schubert J, et al. Genotype-phenotype correlations in SCN8A-related disorders reveal prognostic and therapeutic implications. **Brain**. 2021 Aug 25;awab321. doi: 10.1093/brain/awab321. Online ahead of print.

255. Liu Y, Schubert J, Sonnenberg L, Helbig KL, Hoei-Hansen CE, Koko M, et al. Neuronal mechanisms of mutations in SCN8A causing epilepsy or intellectual disability. **Brain** 142: 376–390, 2019. doi: 10.1093/brain/awy326.

256. Wagnon JL, Barker BS, Ottolini M, Park Y, Volkheimer A, Valdez P, et al. Loss-of-function variants of SCN8A in intellectual disability without seizures. **Neurol Genet** 3: e170, 2017. doi: 10.1212/NXG.0000000000000170.

257. Veeramah KR, O'Brien JE, Meisler MH, Cheng X, Dib-Hajj SD, Waxman SG, et al. De Novo Pathogenic SCN8A Mutation Identified by Whole-Genome Sequencing of a Family Quartet Affected by Infantile Epileptic Encephalopathy and SUDEP. **Am J Hum Genet** 90: 502–510, 2012. doi: 10.1016/j.ajhg.2012.01.006.

258. Wagnon JL, Korn MJ, Parent R, Tarpey TA, Jones JM, Hammer MF, et al. Convulsive seizures and SUDEP in a mouse model of SCN8A epileptic encephalopathy. **Hum Mol Genet** 24: 506–515, 2015. doi: 10.1093/hmg/ddu470.

259. Bunton-Stasyshyn RKA, Wagnon JL, Wengert ER, Barker BS, Faulkner A, Wagley PK, et al. Prominent role of forebrain excitatory neurons in SCN8A encephalopathy. **Brain** 142: 362–375, 2019. doi: 10.1093/brain/awy324.

260. Meisler MH, Plummer NW, Burgess DL, Buchner DA, Sprunger LK. Allelic mutations of the sodium channel SCN8A reveal multiple cellular and physiological functions. **Genetica** 122: 37–45, 2004. doi: 10.1007/s10709-004-1441-9.

261. Boerma RS, Braun KP, van de Broek MPH, van Berkestijn FMC, Swinkels ME, Hagebeuk EO, et al. Remarkable Phenytoin Sensitivity in 4 Children with SCN8A-related Epilepsy: A Molecular Neuropharmacological Approach. **Neurotherapeutics** 13: 192–197, 2016. doi: 10.1007/s13311-015-0372-8.

262. Lenk GM, Jafar-Nejad P, Hill SF, Huffman LD, Smolen CE, Wagnon JL, et al. Scn8a Antisense Oligonucleotide Is Protective in Mouse Models of SCN8A Encephalopathy and Dravet Syndrome. **Ann Neurol** 87: 339–346, 2020. doi: 10.1002/ana.25676.

263. Patino GA, Claes LRF, Lopez-Santiago LF, Slat EA, Dondeti RSR, Chen C, et al. A Functional Null Mutation of SCN1B in a Patient with Dravet Syndrome. **J Neurosci** 29: 10764–10778, 2009. doi: 10.1523/JNEUROSCI.2475-09.2009.

264. Aeby A, Sculier C, Bouza AA, Askar B, Lederer D, Schoonjans A, et al. SCN1B -linked early infantile developmental and epileptic encephalopathy. **Ann Clin Transl Neurol** 6: 2354–2367, 2019. doi: 10.1002/acn3.50921.

265. Scala M, Efthymiou S, Sultan T, De Waele J, Panciroli M, Salpietro V, et al. Homozygous SCN1B variants causing early infantile epileptic encephalopathy 52 affect voltage-gated sodium channel function. **Epilepsia** 62, 2021. doi: 10.1111/epi.16913.

266. Hull JM, O'Malley HA, Chen C, Yuan Y, Denomme N, Bouza AA, et al. Excitatory and inhibitory neuron defects in a mouse model of Scn1b -linked EIEE52. **Ann Clin Transl Neurol** 7: 2137–2149, 2020. doi: 10.1002/acn3.51205.

267. Nappi P, Miceli F, Soldovieri MV, Ambrosino P, Barrese V, Taglialatela M. Epileptic channelopathies caused by neuronal Kv7 (KCNQ) channel dysfunction. **Pflügers Arch** 472:

881–898, 2020. doi: 10.1007/s00424-020-02404-2.

268. Maljevic S, Lerche H. Potassium channel genes and benign familial neonatal epilepsy. In: **Progress in Brain Research**. Elsevier, p. 17–53.

269. Tzingounis A V, Heidenreich M, Kharkovets T, Spitzmaul G, Jensen HS, Nicoll RA, et al. The KCNQ5 potassium channel mediates a component of the afterhyperpolarization current in mouse hippocampus. **Proc Natl Acad Sci** 107: 10232–10237, 2010. doi: 10.1073/pnas.1004644107.

270. Delmas P, Brown DA. Pathways modulating neural KCNQ/M (Kv7) potassium channels. **Nat Rev Neurosci** 6: 850–862, 2005. doi: 10.1038/nrn1785.

271. Weckhuysen S, Mandelstam S, Suls A, Audenaert D, Deconinck T, Claes LRF, et al. KCNQ2 encephalopathy: Emerging phenotype of a neonatal epileptic encephalopathy. **Ann Neurol** 71: 15–25, 2012. doi: 10.1002/ana.22644.

272. Gu N, Vervaeke K, Hu H, Storm JF. Kv7/KCNQ/M and HCN/h, but not K_{Ca} 2/SK channels, contribute to the somatic medium after-hyperpolarization and excitability control in CA1 hippocampal pyramidal cells: M- and h-channel functions in hippocampal pyramidal cells. **J Physiol** 566: 689–715, 2005. doi: 10.1113/jphysiol.2005.086835.

273. Tzingounis A V, Nicoll RA. Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents. **Proc Natl Acad Sci** 105: 19974–19979, 2008. doi: 10.1073/pnas.0810535105.

274. Battefeld A, Tran BT, Gavrilis J, Cooper EC, Kole MHP. Heteromeric Kv7.2/7.3 Channels Differentially Regulate Action Potential Initiation and Conduction in Neocortical Myelinated Axons. **J Neurosci** 34: 3719–3732, 2014. doi: 10.1523/JNEUROSCI.4206-13.2014.

275. Pan Z, Kao T, Horvath Z, Lemos J, Sul J-Y, Cranstoun SD, et al. A Common Ankyrin-G-Based Mechanism Retains KCNQ and Na_V Channels at Electrically Active Domains of the Axon. **J**

Neurosci 26: 2599–2613, 2006. doi: 10.1523/JNEUROSCI.4314-05.2006.

276. Devaux JJ. KCNQ2 Is a Nodal K+ Channel. **J Neurosci** 24: 1236–1244, 2004. doi: 10.1523/JNEUROSCI.4512-03.2004.

277. Martire M, Castaldo P, D'Amico M, Preziosi P, Annunziato L, Taglialatela M. M channels containing KCNQ2 subunits modulate norepinephrine, aspartate, and GABA release from hippocampal nerve terminals. **J Neurosci** 24: 592–597, 2004. doi: 10.1523/JNEUROSCI.3143-03.2004.

278. Peretz A, Sheinin A, Yue C, Degani-Katzav N, Gibor G, Nachman R, et al. Pre- and Postsynaptic Activation of M-Channels By a Novel Opener Dampens Neuronal Firing and Transmitter Release. **J Neurophysiol** 97: 283–295, 2007. doi: 10.1152/jn.00634.2006.

279. Lawrence JJ, Saraga F, Churchill JF, Statland JM, Travis KE, Skinner FK, et al. Somatodendritic Kv7/KCNQ/M Channels Control Interspike Interval in Hippocampal Interneurons. **J Neurosci** 26: 12325–12338, 2006. doi: 10.1523/JNEUROSCI.3521-06.2006.

280. Abidi A, Devaux JJ, Molinari F, Alcaraz G, Michon F-X, Sutera-Sardo J, et al. A recurrent KCNQ2 pore mutation causing early onset epileptic encephalopathy has a moderate effect on M current but alters subcellular localization of Kv7 channels. **Neurobiol Dis** 80: 80–92, 2015. doi: 10.1016/j.nbd.2015.04.017.

281. Milh M, Roubertoux P, Biba N, Chavany J, Spiga Ghata A, Fulachier C, et al. A knock-in mouse model for *KCNQ2* -related epileptic encephalopathy displays spontaneous generalized seizures and cognitive impairment. **Epilepsia** 61: 868–878, 2020. doi: 10.1111/epi.16494.

282. Orhan G, Bock M, Schepers D, Ilina EI, Reichel SN, Löffler H, et al. Dominant-negative effects of *KCNQ2* mutations are associated with epileptic encephalopathy: *KCNQ2* Defects in EE. **Ann Neurol** 75: 382–394, 2014. doi: 10.1002/ana.24080.

283. Soh H, Park S, Ryan K, Springer K, Maheshwari A, Tzingounis A V. Deletion of KCNQ2/3 potassium channels from PV+ interneurons leads to homeostatic potentiation of excitatory transmission. **Elife** 7: e38617, 2018. doi: 10.7554/eLife.38617.

284. Wang H, Kunkel D, Schwartzkroin P, Tempel B. Localization of Kv1.1 and Kv1.2, two K channel proteins, to synaptic terminals, somata, and dendrites in the mouse brain. **J Neurosci** 14: 4588–4599, 1994. doi: 10.1523/JNEUROSCI.14-08-04588.1994.

285. Monaghan MM, Trimmer JS, Rhodes KJ. Experimental Localization of Kv1 Family Voltage-Gated K $^{+}$ Channel $α$ and $β$ Subunits in Rat Hippocampal Formation. **J Neurosci** 21: 5973–5983, 2001. doi: 10.1523/JNEUROSCI.21-16-05973.2001.

286. Lorincz A, Nusser Z. Cell-Type-Dependent Molecular Composition of the Axon Initial Segment. **J Neurosci** 28: 14329–14340, 2008. doi: 10.1523/JNEUROSCI.4833-08.2008.

287. D'Adamo MC, Imbrici P, Sponcichetti F, Pessia M. Mutations in the KCNA1 gene associated with episodic ataxia type-1 syndrome impair heteromeric voltage-gated K(+) channel function. **FASEB J** 13: 1335–1345, 1999. doi: 10.1096/fasebj.13.11.1335.

288. Hivert B, Marien L, Agbam KN, Faivre-Sarrailh C. ADAM22 and ADAM23 modulate the targeting of the Kv1 channel-associated protein LGI1 to the axon initial segment. **J Cell Sci** 132: jcs219774, 2019. doi: 10.1242/jcs.219774..

289. Pena SDJ, Coimbra RLM. Ataxia and myoclonic epilepsy due to a heterozygous new mutation in *KCNA2* : proposal for a new channelopathy: Ataxia and myoclonic epilepsy due to a heterozygous new mutation. **Clin Genet** 87: e1--e3, 2015. doi: 10.1111/cge.12542.

290. Masnada S, Hedrich UBS, Gardella E, Schubert J, Kaiwar C, Klee EW, et al. Clinical spectrum and genotype–phenotype associations of KCNA2-related encephalopathies. **Brain** 140: 2337–2354, 2017. doi: 10.1093/brain/awx184.

291. Brew HM, Gittelman JX, Silverstein RS, Hanks TD, Demas VP, Robinson LC, et al. Seizures and

Reduced Life Span in Mice Lacking the Potassium Channel Subunit Kv1.2, but Hypoexcitability and Enlarged Kv1 Currents in Auditory Neurons. **J Neurophysiol** 98: 1501–1525, 2007. doi: 10.1152/jn.00640.2006.

292. Xie G, Harrison J, Clapcote SJ, Huang Y, Zhang J-Y, Wang L-Y, et al. A new Kv1.2 channelopathy underlying cerebellar ataxia. **J Biol Chem** 285: 32160–32173, 2010. doi: 10.1074/jbc.M110.153676.

293. Hedrich UBS, Lauxmann S, Wolff M, Synofzik M, Bast T, Binelli A, et al. 4-Aminopyridine is a promising treatment option for patients with gain-of-function *KCNA2* -encephalopathy. **Sci Transl Med** 13: eaaz4957, 2021. doi: 10.1126/scitranslmed.aaz4957.

294. Rogers A, Golumbek P, Cellini E, Doccini V, Guerrini R, Wallgren-Pettersson C, et al. De novo *KCNA1* variants in the PVP motif cause infantile epileptic encephalopathy and cognitive impairment similar to recurrent *KCNA2* variants. **Am J Med Genet Part A** 176: 1748–1752, 2018. doi: 10.1002/ajmg.a.38840.

295. Verdura E, Fons C, Schlüter A, Ruiz M, Fourcade S, Casasnovas C, et al. Complete loss of KCNA1 activity causes neonatal epileptic encephalopathy and dyskinesia. **J Med Genet** 57: 132–137, 2020. doi: 10.1136/jmedgenet-2019-106373.

296. Trimmer JSS. Subcellular Localization of K+ Channels in Mammalian Brain Neurons: Remarkable Precision in the Midst of Extraordinary Complexity. **Neuron** 85: 238–256, 2015. doi: 10.1016/j.neuron.2014.12.042.

297. Mohapatra DP, Park K-S, Trimmer JS. Dynamic regulation of the voltage-gated Kv2.1 potassium channel by multisite phosphorylation. **Biochem Soc Trans** 35: 1064–1068, 2007. doi: 10.1042/BST0351064.

298. Speca DJ, Ogata G, Mandikian D, Bishop HI, Wiler SW, Eum K, et al. Deletion of the Kv2.1 delayed rectifier potassium channel leads to neuronal and behavioral hyperexcitability:

Kv2.1 deletion and hyperexcitability. **Genes, Brain Behav** 13: 394–408, 2014. doi: 10.1111/gbb.12120.

299. Torkamani A, Bersell K, Jorge BS, Bjork RL, Friedman JR, Bloss CS, et al. De novo KCNB1 mutations in epileptic encephalopathy. **Ann Neurol** 76: 529–540, 2014. doi: 10.1002/ana.24263.

300. Marini C, Romoli M, Parrini E, Costa C, Mei D, Mari F, et al. Clinical features and outcome of 6 new patients carrying de novo KCNB1 gene mutations. **Neurol Genet** 3: e206, 2017. doi: 10.1212/NXG.0000000000000206.

301. Bar C, Barcia G, Jennesson M, Le Guyader G, Schneider A, Mignot C, et al. Expanding the genetic and phenotypic relevance of KCNB1 variants in developmental and epileptic encephalopathies: 27 new patients and overview of the literature. **Hum Mutat** 41: 69–80, 2020. doi: 10.1002/humu.23915.

302. Thiffault I, Speca DJ, Austin DC, Cobb MM, Eum KS, Safina NP, et al. A novel epileptic encephalopathy mutation in KCNB1 disrupts Kv2.1 ion selectivity, expression, and localization. **J Gen Physiol** 146: 399–410, 2015. doi: 10.1085/jgp.201511444.

303. Calhoun JD, Vanoye CG, Kok F, George AL, Kearney JA. Characterization of a KCNB1 variant associated with autism, intellectual disability, and epilepsy. **Neurol Genet** 3: e198, 2017. doi: 10.1212/NXG.0000000000000198.

304. Czirják G, Tóth ZE, Enyedi P. Characterization of the heteromeric potassium channel formed by Kv2.1 and the retinal subunit Kv8.2 in Xenopus oocytes. **J Neurophysiol** 98: 1213–1222, 2007. doi: 10.1152/jn.00493.2007.

305. Jorge BS, Campbell CM, Miller AR, Rutter ED, Gurnett CA, Vanoye CG, et al. Voltage-gated potassium channel KCNV2 (Kv8.2) contributes to epilepsy susceptibility. **Proc Natl Acad Sci** 108: 5443–5448, 2011. doi: 10.1073/pnas.1017539108.

306. Kaczmarek LK, Zhang Y. Kv3 Channels: Enablers of Rapid Firing, Neurotransmitter Release, and Neuronal Endurance. **Physiol Rev** 97: 1431–1468, 2017. doi: 10.1152/physrev.00002.2017.

307. Xu M, Cao R, Xiao R, Zhu MX, Gu C. The Axon Dendrite Targeting of Kv3 (Shaw) Channels Is Determined by a Targeting Motif That Associates with the T1 Domain and Ankyrin G. **J Neurosci** 27: 14158–14170, 2007. doi: 10.1523/JNEUROSCI.3675-07.2007.

308. Rudy B, McBain CJ. Kv3 channels: voltage-gated K+ channels designed for high-frequency repetitive firing. **Trends Neurosci** 24: 517–526, 2001. doi: 10.1016/S0166-2236(00)01892-0.

309. Cameron JM, Maljevic S, Nair U, Aung YH, Cogné B, Bézieau S, et al. Encephalopathies with KCNC1 variants: genotype-phenotype-functional correlations. **Ann Clin Transl Neurol** 6: 1263–1272, 2019. doi: 10.1002/acn3.50822.

310. Park J, Koko M, Hedrich UBS, Hermann A, Cremer K, Haberlandt E, et al. KCNC1 -related disorders: new de novo variants expand the phenotypic spectrum. **Ann Clin Transl Neurol** 6: 1319–1326, 2019. doi: 10.1002/acn3.50799.

311. Muona M, Berkovic SF, Dibbens LM, Oliver KL, Maljevic S, Bayly MA, et al. A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. **Nat Genet** 47: 39–46, 2015. doi: 10.1038/ng.3144.

312. Espinosa F, McMahon A, Chan E, Wang S, Ho CS, Heintz N, et al. Alcohol Hypersensitivity, Increased Locomotion, and Spontaneous Myoclonus in Mice Lacking the Potassium Channels Kv3.1 and Kv3.3. **J Neurosci** 21: 6657–6665, 2001. doi: 10.1523/JNEUROSCI.21-17-06657.2001.

313. Isbrandt D, Leicher T, Waldschütz R, Zhu X, Luhmann U, Michel U, et al. Gene Structures and Expression Profiles of Three Human KCND (Kv4) Potassium Channels Mediating A-Type Currents ITO and ISA. **Genomics** 64: 144–154, 2000. doi: 10.1006/geno.2000.6117.

314. Lee H, Lin MA, Kornblum HI, Papazian DM, Nelson SF. Exome sequencing identifies de novo gain of function missense mutation in KCND2 in identical twins with autism and seizures that slows potassium channel inactivation. **Hum Mol Genet** 23: 3481–3489, 2014. doi: 10.1093/hmg/ddu056.

315. Schönherr R, Gessner G, Löber K, Heinemann SH. Functional distinction of human EAG1 and EAG2 potassium channels. **FEBS Lett** 514: 204–208, 2002. doi: 10.1016/S0014-5793(02)02365-7.

316. Kortüm F, Caputo V, Bauer CK, Stella L, Ciolfi A, Alawi M, et al. Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome. **Nat Genet** 47: 661–667, 2015. doi: 10.1038/ng.3282.

317. Simons C, Rash LD, Crawford J, Ma L, Cristofori-Armstrong B, Miller D, et al. Mutations in the voltage-gated potassium channel gene KCNH1 cause Temple-Baraitser syndrome and epilepsy. **Nat Genet** 47: 73–77, 2015. doi: 10.1038/ng.3153.

318. Bramswig NC, Ockeloen CW, Czeschik JC, van Essen AJ, Pfundt R, Smeitink J, et al. 'Splitting versus lumping': Temple–Baraitser and Zimmermann–Laband Syndromes. **Hum Genet** 134: 1089–1097, 2015. doi: 10.1007/s00439-015-1590-1.

319. Mastrangelo M, Scheffer IE, Bramswig NC, Nair LD V, Myers CT, Dentici ML, et al. Epilepsy in KCNH1-related syndromes. **Epileptic Disord** 18: 123–136, 2016. doi: 10.1684/epd.2016.0830.

320. von Wrede R, Jeub M, Ariöz I, Elger CE, von Voss H, Klein H-G, et al. Novel KCNH1 Mutations Associated with Epilepsy: Broadening the Phenotypic Spectrum of KCNH1-Associated Diseases. **Genes** 12: 132, 2021. doi: 10.3390/genes12020132.

321. Veeramah KR, Johnstone L, Karafet TM, Wolf D, Sprissler R, Salogiannis J, et al. Exome sequencing reveals new causal mutations in children with epileptic encephalopathies.

Epilepsia 54: 1270–1281, 2013. doi: 10.1111/epi.12201.

322. Yang Y, Vasylyev D V, Dib-Hajj F, Veeramah KR, Hammer MF, Dib-Hajj SD, et al. Multistate Structural Modeling and Voltage-Clamp Analysis of Epilepsy/Autism Mutation Kv10.2-R327H Demonstrate the Role of This Residue in Stabilizing the Channel Closed State. **J Neurosci** 33: 16586–16593, 2013. doi: 10.1523/JNEUROSCI.2307-13.2013.

323. Gonzalez-Perez V, Lingle CJ. Regulation of BK Channels by Beta and Gamma Subunits. **Annu Rev Physiol** 81: 113–137, 2019. doi: 10.1146/annurev-physiol-022516-034038.

324. Zhang Z-B, Tian M-Q, Gao K, Jiang Y-W, Wu Y. De novo KCNMA1 mutations in children with early-onset paroxysmal dyskinesia and developmental delay. **Mov Disord** 30: 1290–1292, 2015. doi: 10.1002/mds.26216.

325. Miller JP, Moldenhauer HJ, Keros S, Meredith AL. An emerging spectrum of variants and clinical features in KCNMA1 -linked channelopathy. **Channels** 15: 447–464, 2021. doi: 10.1080/19336950.2021.1938852.

326. Liang L, Li X, Moutton S, Schrier Vergano SA, Cogné B, Saint-Martin A, et al. De novo loss-of-function KCNMA1 variants are associated with a new multiple malformation syndrome and a broad spectrum of developmental and neurological phenotypes. **Hum Mol Genet** 28: 2937–2951, 2019. doi: 10.1093/hmg/ddz117.

327. Meredith AL, Thorneloe KS, Werner ME, Nelson MT, Aldrich RW. Overactive Bladder and Incontinence in the Absence of the BK Large Conductance Ca2+-activated K+ Channel. **J Biol Chem** 279: 36746–36752, 2004. doi: 10.1074/jbc.M405621200.

328. Typlt M, Mirkowski M, Azzopardi E, Ruettiger L, Ruth P, Schmid S. Mice with Deficient BK Channel Function Show Impaired Prepulse Inhibition and Spatial Learning, but Normal Working and Spatial Reference Memory. **PLoS One** 8: e81270, 2013. doi: 10.1371/journal.pone.0081270.

329. Imlach WL, Finch SC, Dunlop J, Meredith AL, Aldrich RW, Dalziel JE. The Molecular Mechanism of “Ryegrass Staggers,” a Neurological Disorder of K + Channels. **J Pharmacol Exp Ther** 327: 657–664, 2008. doi: 10.1124/jpet.108.143933.

330. Rizzi S, Knaus H-G, Schwarzer C. Differential distribution of the sodium-activated potassium channels slick and slack in mouse brain. **J Comp Neurol** 524: 2093–2116, 2016. doi: 10.1002/cne.23934.

331. Mao X, Bruneau N, Gao Q, Becq H, Jia Z, Xi H, et al. The Epilepsy of Infancy With Migrating Focal Seizures: Identification of de novo Mutations of the KCNT2 Gene That Exert Inhibitory Effects on the Corresponding Heteromeric KNa1.1/KNa1.2 Potassium Channel. **Front Cell Neurosci** 14, 2020. doi: 10.3389/fncel.2020.00001.

332. Franceschetti S, Lavazza T, Curia G, Aracri P, Panzica F, Sancini G, et al. Na + -Activated K + Current Contributes to Postexcitatory Hyperpolarization in Neocortical Intrinsically Bursting Neurons. **J Neurophysiol** 89: 2101–2111, 2003. doi: 10.1152/jn.00695.2002.

333. Barcia G, Fleming MR, Deligniere A, Gazula V-R, Brown MR, Langouet M, et al. De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. **Nat Genet** 44: 1255–1259, 2012. doi: 10.1038/ng.2441.

334. Heron SE, Smith KR, Bahlo M, Nobili L, Kahana E, Licchetta L, et al. Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. **Nat Genet** 44: 1188–1190, 2012. doi: 10.1038/ng.2440.

335. Cole BA, Clapcote SJ, Muench SP, Lippiat JD. Targeting KNa1.1 channels in KCNT1-associated epilepsy. **Trends Pharmacol Sci** 42: 700–713, 2021. doi: 10.1016/j.tips.2021.05.003.

336. Kim GE, Kronengold J, Barcia G, Quraishi IH, Martin HC, Blair E, et al. Human Slack Potassium Channel Mutations Increase Positive Cooperativity between Individual Channels. **Cell Rep** 9: 1661–1672, 2014. doi: 10.1016/j.celrep.2014.11.015.

337. Evely KM, Pryce KD, Bhattacharjee A. The Phe932Ile mutation in KCNT1 channels associated with severe epilepsy, delayed myelination and leukoencephalopathy produces a loss-of-function channel phenotype. **Neuroscience** 351: 65–70, 2017. doi: 10.1016/j.neuroscience.2017.03.035.

338. Shore AN, Colombo S, Tobin WF, Petri S, Cullen ER, Dominguez S, et al. Reduced GABAergic Neuron Excitability, Altered Synaptic Connectivity, and Seizures in a KCNT1 Gain-of-Function Mouse Model of Childhood Epilepsy. **Cell Rep** 33: 108303, 2020. doi: 10.1016/j.celrep.2020.108303.

339. Ambrosino P, Soldovieri MV, Bast T, Turnpenny PD, Uhrig S, Biskup S, et al. De novo gain-of-function variants in KCNT2 as a novel cause of developmental and epileptic encephalopathy. **Ann Neurol** 83: 1198–1204, 2018. doi: 10.1002/ana.25248.

340. Simms BA, Zamponi GW. Neuronal Voltage-Gated Calcium Channels: Structure, Function, and Dysfunction. **Neuron** 82: 24–45, 2014. doi: 10.1016/j.neuron.2014.03.016.

341. Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. Voltage-gated calcium channels in GtoPdb v.2021.2. **IUPHAR/BPS Guid to Pharmacol CITE** 2021, 2021. doi: 10.2218/gtopdb/F80/2021.2.

342. Laryushkin DP, Maiorov SA, Zinchenko VP, Gaidin SG, Kosenkov AM. Role of l-type voltage-gated calcium channels in epileptiform activity of neurons. **Int J Mol Sci** 22, 2021. doi: 10.3390/ijms221910342.

343. Striessnig J, Ortner N, Pinggera A. Pharmacology of L-type Calcium Channels: Novel Drugs for Old Targets? **Curr Mol Pharmacol** 8: 110–122, 2015. doi: 10.2174/1874467208666150507105845.

344. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. Ca V 1.2 Calcium Channel Dysfunction Causes a Multisystem Disorder Including Arrhythmia and Autism

ultimate signaling molecule for organisms ranging from prokaryotes to humans. In higher organisms, Ca 2 medi-ates processes as diverse as synaptic transmiss. **Cell** 119: 19–31, 2004.

345. Campiglio M, Flucher BE. The role of auxiliary subunits for the functional diversity of voltage-gated calcium channels. **J Cell Physiol** 230: 2019–2031, 2015. doi: 10.1002/jcp.24998.

346. Perez-Reyes E. Molecular Physiology of Low-Voltage-Activated T-type Calcium Channels. **Physiol Rev** 83: 117–161, 2003. doi: 10.1152/physrev.00018.2002.

347. Crandall SR, Govindaiah G, Cox CL. Low-Threshold Ca2+ Current Amplifies Distal Dendritic Signaling in Thalamic Reticular Neurons. **J Neurosci** 30: 15419–15429, 2010. doi: 10.1523/JNEUROSCI.3636-10.2010.

348. Tsakiridou E, Bertollini L, de Curtis M, Avanzini G, Pape H. Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. **J Neurosci** 15: 3110–3117, 1995. doi: 10.1523/JNEUROSCI.15-04-03110.1995.

349. Lory P, Nicole S, Monteil A. Neuronal Cav3 channelopathies: recent progress and perspectives. **Pflügers Arch** 472: 831–844, 2020. doi: 10.1007/s00424-020-02429-7.

350. Kunii M, Doi H, Hashiguchi S, Matsuishi T, Sakai Y, Iai M, et al. De novo CACNA1G variants in developmental delay and early-onset epileptic encephalopathies. **J Neurol Sci** 416: 117047, 2020. doi: 10.1016/j.jns.2020.117047.

351. Berecki G, Helbig KL, Ware TL, Grinton B, Skraban CM, Marsh ED, et al. Novel missense cacna1g mutations associated with infantile-onset developmental and epileptic encephalopathy. **Int J Mol Sci** 21: 1–15, 2020. doi: 10.3390/ijms21176333.

352. El Ghaleb Y, Schneeberger PE, Fernández-Quintero ML, Geisler SM, Pelizzari S, Polstra AM, et al. CACNA1I gain-of-function mutations differentially affect channel gating and cause neurodevelopmental disorders. **Brain** 144: 2092–2106, 2021. doi: 10.1093/brain/awab101.

353. Dolphin AC, Lee A. Presynaptic calcium channels: specialized control of synaptic neurotransmitter release. **Nat Rev Neurosci** 21: 213–229, 2020. doi: 10.1038/s41583-020-0278-2.

354. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SMG, et al. Familial Hemiplegic Migraine and Episodic Ataxia Type-2 Are Caused by Mutations in the Ca2+ Channel Gene CACNL1A4. **Cell** 87: 543–552, 1996. doi: 10.1016/S0092-8674(00)81373-2.

355. Catterall WA, Lenaeus MJ, Gamal El-Din TM. Structure and Pharmacology of Voltage-Gated Sodium and Calcium Channels. **Annu Rev Pharmacol Toxicol** 60: 133–154, 2020. doi: 10.1146/annurev-pharmtox-010818-021757.

356. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, et al. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α1A-voltage-dependent calcium channel. **Nat Genet** 15: 62–69, 1997. doi: 10.1038/ng0197-62.

357. Indelicato E, Boesch S. From Genotype to Phenotype: Expanding the Clinical Spectrum of CACNA1A Variants in the Era of Next Generation Sequencing. **Front Neurol** 12, 2021. doi: 10.3389/fneur.2021.639994.

358. Jiang X, Raju PK, D'Avanzo N, Lachance M, Pepin J, Dubeau F, et al. Both gain-of-function and loss-of-function de novo CACNA1A mutations cause severe developmental epileptic encephalopathies in the spectrum of Lennox-Gastaut syndrome. **Epilepsia** 60: 1881–1894, 2019. doi: 10.1111/epi.16316.

359. Gorman KM, Meyer E, Grozeva D, Spinelli E, McTague A, Sanchis-Juan A, et al. Bi-allelic Loss-of-Function CACNA1B Mutations in Progressive Epilepsy-Dyskinesia. **Am J Hum Genet** 104: 948–956, 2019. doi: 10.1016/j.ajhg.2019.03.005.

360. Nakagawasai O, Onogi H, Mitazaki S, Sato A, Watanabe K, Saito H, et al. Behavioral and

neurochemical characterization of mice deficient in the N-type Ca2+ channel α1B subunit. **Behav Brain Res** 208: 224–230, 2010. doi: 10.1016/j.bbr.2009.11.042.

361. Helbig KL, Lauerer RJ, Bahr JC, Souza IA, Myers CT, Uysal B, et al. De Novo Pathogenic Variants in CACNA1E Cause Developmental and Epileptic Encephalopathy with Contractures, Macrocephaly, and Dyskinesias. **Am J Hum Genet** 103: 666–678, 2018. doi: 10.1016/j.ajhg.2018.09.006.

362. Saegusa H, Kurihara T, Zong S, Minowa O, Kazuno A–., Han W, et al. Altered pain responses in mice lacking alpha 1E subunit of the voltage-dependent Ca2+ channel. **Proc Natl Acad Sci** 97: 6132–6137, 2000. doi: 10.1073/pnas.100124197.

363. Lu B, Su Y, Das S, Liu J, Xia J, Ren D. The neuronal channel NALCN contributes resting sodium permeability and is required for normal respiratory rhythm. **Cell** 129: 371–383, 2007. doi: 10.1016/j.cell.2007.02.041.

364. Cochet-Bissuel M, Lory P, Monteil A. The sodium leak channel, NALCN, in health and disease. **Front Cell Neurosci** 8, 2014. doi: 10.3389/fncel.2014.00132.

365. Al-Sayed MD, Al-Zaidan H, Albakheet A, Hakami H, Kenana R, Al-Yafee Y, et al. Mutations in NALCN Cause an Autosomal-Recessive Syndrome with Severe Hypotonia, Speech Impairment, and Cognitive Delay. **Am J Hum Genet** 93: 721–726, 2013. doi: 10.1016/j.ajhg.2013.08.001.

366. Bramswig NC, Bertoli-Avella AM, Albrecht B, Al Aqeel AI, Alhashem A, Al-Sannaa N, et al. Genetic variants in components of the NALCN–UNC80–UNC79 ion channel complex cause a broad clinical phenotype (NALCN channelopathies). **Hum Genet** 137: 753–768, 2018. doi: 10.1007/s00439-018-1929-5.

367. Chong JX, McMillin MJ, Shively KM, Beck AE, Marvin CT, Armenteros JR, et al. De Novo Mutations in NALCN Cause a Syndrome Characterized by Congenital Contractures of the

Limbs and Face, Hypotonia, and Developmental Delay. **Am J Hum Genet** 96: 462–473, 2015. doi: 10.1016/j.ajhg.2015.01.003.

368. Bouasse M, Impheng H, Servant Z, Lory P, Monteil A. Functional expression of CLIFAHDD and IHPRF pathogenic variants of the NALCN channel in neuronal cells reveals both gain- and loss-of-function properties. **Sci Rep** 9: 11791, 2019. doi: 10.1038/s41598-019-48071-x.

369. Rivolta I, Binda A, Masi A, DiFrancesco JC. Cardiac and neuronal HCN channelopathies. **Pflügers Arch** 472: 931–951, 2020. doi: 10.1007/s00424-020-02384-3.

370. Combe CL, Gasparini S. Ih from synapses to networks: HCN channel functions and modulation in neurons. **Prog Biophys Mol Biol** 166: 119–132, 2021. doi: 10.1016/j.pbiomolbio.2021.06.002.

371. Nava C, Dalle C, Rastetter A, Striano P, de Kovel CGF, Nabbout R, et al. De novo mutations in HCN1 cause early infantile epileptic encephalopathy. **Nat Genet** 46: 640–645, 2014. doi: 10.1038/ng.2952.

372. Marini C, Porro A, Rastetter A, Dalle C, Rivolta I, Bauer D, et al. HCN1 mutation spectrum: from neonatal epileptic encephalopathy to benign generalized epilepsy and beyond. **Brain** 141: 3160–3178, 2018. doi: 10.1093/brain/awy263.

373. Nolan MF, Malleret G, Lee KH, Gibbs E, Dudman JT, Santoro B, et al. The Hyperpolarization-Activated HCN1 Channel Is Important for Motor Learning and Neuronal Integration by Cerebellar Purkinje Cells. **Cell** 115: 551–564, 2003. doi: 10.1016/S0092-8674(03)00884-5.

374. Bleakley LE, McKenzie CE, Soh MS, Forster IC, Pinares-Garcia P, Sedo A, et al. Cation leak underlies neuronal excitability in an HCN1 developmental and epileptic encephalopathy. **Brain** 144: 2060–2073, 2021. doi: 10.1093/brain/awab145.

375. Friedrich T, Tavraz NN, Junghans C. ATP1A2 Mutations in Migraine: Seeing through the Facets of an Ion Pump onto the Neurobiology of Disease. **Front Physiol** 7, 2016. doi:

10.3389/fphys.2016.00239.

376. Kaplan JH. Biochemistry of Na,K-ATPase. **Annu Rev Biochem** 71: 511–535, 2002. doi: 10.1146/annurev.biochem.71.102201.141218.

377. Bassi MT. A novel mutation in the ATP1A2 gene causes alternating hemiplegia of childhood. **J Med Genet** 41: 621–628, 2004. doi: 10.1136/jmg.2003.017863.

378. Swoboda KJ, Kanavakis E, Xaidara A, Johnson JE, Leppert MF, Schlesinger-Massart MB, et al. Alternating hemiplegia of childhood or familial hemiplegic migraine?: A novelATP1A2 mutation. **Ann Neurol** 55: 884–887, 2004. doi: 10.1002/ana.20134.

379. Dard R, Mignot C, Durr A, Lesca G, Sanlaville D, Roze E, et al. Relapsing encephalopathy with cerebellar ataxia related to an ATP1A3 mutation. **Dev Med Child Neurol** 57: 1183–1186, 2015. doi: 10.1111/dmcn.12927.

380. Paciorkowski AR, McDaniel SS, Jansen LA, Tully H, Tuttle E, Ghoneim DH, et al. Novel mutations in ATP1A3 associated with catastrophic early life epilepsy, episodic prolonged apnea, and postnatal microcephaly. **Epilepsia** 56: 422–430, 2015. doi: 10.1111/epi.12914.

381. Liu J, Tong L, Song S, Niu Y, Li J, Wu X, et al. Novel and de novo mutations in pediatric refractory epilepsy. **Mol Brain** 11: 48, 2018. doi: 10.1186/s13041-018-0392-5.

382. Chatron N, Cabet S, Alix E, Buenerd A, Cox P, Guibaud L, et al. A novel lethal recognizable polymicrogyric syndrome caused by ATP1A2 homozygous truncating variants. **Brain** 142: 3367–3374, 2019. doi: 10.1093/brain/awz272.

383. Monteiro FP, Curry CJ, Hevner R, Elliott S, Fisher JH, Turocy J, et al. Biallelic loss of function variants in ATP1A2 cause hydrops fetalis, microcephaly, arthrogryposis and extensive cortical malformations. **Eur J Med Genet** 63: 103624, 2020. doi: 10.1016/j.ejmg.2019.01.014.

384. Vetro A, Nielsen HN, Holm R, Hevner RF, Parrini E, Powis Z, et al. ATP1A2- and ATP1A3-

associated early profound epileptic encephalopathy and polymicrogyria. **Brain** 144: 1435–1450, 2021. doi: 10.1093/brain/awab052.

385. Sterlini B, Romei A, Parodi C, Aprile D, Oneto M, Aperia A, et al. An interaction between PRRT2 and Na+/K+ ATPase contributes to the control of neuronal excitability. **Cell Death Dis** 12: 292, 2021. doi: 10.1038/s41419-021-03569-z.

386. Chanaday NL, Cousin MA, Milosevic I, Watanabe S, Morgan JR. The Synaptic Vesicle Cycle Revisited: New Insights into the Modes and Mechanisms. **J Neurosci** 39: 8209–8216, 2019. doi: 10.1523/JNEUROSCI.1158-19.2019.

387. Fassio A, Fadda M, Benfenati F. Molecular Machines Determining the Fate of Endocytosed Synaptic Vesicles in Nerve Terminals. **Front Synaptic Neurosci** 8: 10, 2016. doi: 10.3389/fnsyn.2016.00010.

388. Alabi AA, Tsien RW. Synaptic vesicle pools and dynamics. **Cold Spring Harb Perspect Biol** 4: a013680, 2012. doi: 10.1101/cshperspect.a013680.

389. Rizzoli SO. Synaptic vesicle recycling: steps and principles. **EMBO J** 33: 788–822, 2014. doi: 10.1002/embj.201386357.

390. Zucker RS, Regehr WG. Short-Term Synaptic Plasticity. **Annu Rev Physiol** 64: 355–405, 2002. doi: 10.1146/annurev.physiol.64.092501.114547.

391. Abbott LF, Regehr WG. Synaptic computation. **Nature** 431: 796–803, 2004. doi: 10.1038/nature03010.

392. Goldberg EM, Coulter DA. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. **Nat Rev Neurosci** 14: 337–349, 2013. doi: 10.1038/nrn3482.

393. Fukata Y, Fukata M. Epilepsy and synaptic proteins. **Curr Opin Neurobiol** 45: 1–8, 2017. doi: 10.1016/j.conb.2017.02.001.

394. Wolking S, May P, Mei D, Møller RS, Balestrini S, Helbig KL, et al. Clinical spectrum of *STX1B*

-related epileptic disorders. Neurology 92: e1238-e1249, 2019. doi: 10.1212/WNL.0000000000007089.

395. Fukuda H, Imagawa E, Hamanaka K, Fujita A, Mitsuhashi S, Miyatake S, et al. A novel missense SNAP25b mutation in two affected siblings from an Israeli family showing seizures and cerebellar ataxia. **J Hum Genet** 63: 673–676, 2018. doi: 10.1038/s10038-018-0421-3.

396. Salpietro V, Malintan NT, Llano-Rivas I, Spaeth CG, Efthymiou S, Striano P, et al. Mutations in the Neuronal Vesicular SNARE VAMP2 Affect Synaptic Membrane Fusion and Impair Human Neurodevelopment. **Am J Hum Genet** 104: 721–730, 2019. doi: 10.1016/j.ajhg.2019.02.016.

397. Stamberger H, Nikanorova M, Willemsen MH, Accorsi P, Angriman M, Baier H, et al. STXBP1 encephalopathy: A neurodevelopmental disorder including epilepsy. **Neurology** 86: 954–962, 2016. doi: 10.1212/WNL.0000000000002457.

398. Valtorta F, Benfenati F, Zara F, Meldolesi J. PRRT2: from Paroxysmal Disorders to Regulation of Synaptic Function. **Trends Neurosci** 39: 668–679, 2016. doi: 10.1016/j.tins.2016.08.005.

399. Baker K, Gordon SL, Melland H, Bumbak F, Scott DJ, Jiang TJ, et al. SYT1-associated neurodevelopmental disorder: a case series. **Brain A J Neurol** 141: 2576–2591, 2018. doi: 10.1093/brain/awy209.

400. Bradberry MM, Courtney NA, Dominguez MJ, Lofquist SM, Knox AT, Sutton RB, et al. Molecular Basis for Synaptotagmin-1-Associated Neurodevelopmental Disorder. **Neuron** 107: 52--64.e7, 2020. doi: 10.1016/j.neuron.2020.04.003.

401. Washbourne P, Thompson PM, Carta M, Costa ET, Mathews JR, Lopez-Benditó G, et al. Genetic ablation of the t-SNARE SNAP-25 distinguishes mechanisms of neuroexocytosis. **Nat Neurosci** 5: 19–26, 2002. doi: 10.1038/nn783.

402. Gerber SH, Rah J-C, Min S-W, Liu X, de Wit H, Dulubova I, et al. Conformational switch of

syntaxin-1 controls synaptic vesicle fusion. **Science** 321: 1507–1510, 2008. doi: 10.1126/science.1163174.

403. Schoch S, Deák F, Königstorfer A, Mozhayeva M, Sara Y, Südhof TC, et al. SNARE function analyzed in synaptobrevin/VAMP knockout mice. **Science** 294: 1117–1122, 2001. doi: 10.1126/science.1064335.

404. Kovacevic J, Maroteaux G, Schut D, Loos M, Dubey M, Pitsch J, et al. Protein instability, haploinsufficiency, and cortical hyper-excitability underlie STXBP1 encephalopathy. **Brain** 141: 1350–1374, 2018. doi: 10.1093/brain/awy046.

405. Michetti C, Castroflorio E, Marchionni I, Forte N, Sterlini B, Binda F, et al. The PRRT2 knockout mouse recapitulates the neurological diseases associated with PRRT2 mutations. **Neurobiol Dis** 99: 66–83, 2017. doi: 10.1016/j.nbd.2016.12.018.

406. Valente P, Romei A, Fadda M, Sterlini B, Lonardoni D, Forte N, et al. Constitutive Inactivation of the PRRT2 Gene Alters Short-Term Synaptic Plasticity and Promotes Network Hyperexcitability in Hippocampal Neurons. **Cereb Cortex** 29: 2010–2033, 2019. doi: 10.1093/cercor/bhy079.

407. Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, et al. Synaptotagmin I: a major Ca2+ sensor for transmitter release at a central synapse. **Cell** 79: 717–727, 1994. doi: 10.1016/0092-8674(94)90556-8.

408. Rohena L, Neidich J, Truitt Cho M, Gonzalez KD, Tang S, Devinsky O, et al. Mutation in SNAP25 as a novel genetic cause of epilepsy and intellectual disability. **Rare Dis** 1: e26314, 2013. doi: 10.4161/rdis.26314.

409. Shen X-M, Selcen D, Brengman J, Engel AG. Mutant SNAP25B causes myasthenia, cortical hyperexcitability, ataxia, and intellectual disability. **Neurology** 83: 2247–2255, 2014. doi: 10.1212/WNL.0000000000001079.

410. Johansson JU, Ericsson J, Janson J, Beraki S, Stanić D, Mandic SA, et al. An ancient duplication of exon 5 in the Snap25 gene is required for complex neuronal development/function. **PLoS Genet** 4: e1000278, 2008. doi: 10.1371/journal.pgen.1000278.

411. Schubert J, Siekierska A, Langlois M, May P, Huneau C, Becker F, et al. Mutations in STX1B, encoding a presynaptic protein, cause fever-associated epilepsy syndromes. **Nat Genet** 46: 1327–1332, 2014. doi: 10.1038/ng.3130.

412. Megighian A, Pirazzini M, Fabris F, Rossetto O, Montecucco C. Tetanus and tetanus neurotoxin: From peripheral uptake to central nervous tissue targets. **J Neurochem** 158: 1244–1253, 2021. doi: 10.1111/jnc.15330.

413. Rizo J. Mechanism of neurotransmitter release coming into focus. **Protein Sci** 27: 1364–1391, 2018. doi: 10.1002/pro.3445.

414. Brunger AT, Choi UB, Lai Y, Leitz J, White KI, Zhou Q. The pre-synaptic fusion machinery. **Curr Opin Struct Biol** 54: 179–188, 2019. doi: 10.1016/j.sbi.2019.03.007.

415. Baker K, Gordon SL, Grozeva D, van Kogelenberg M, Roberts NY, Pike M, et al. Identification of a human synaptotagmin-1 mutation that perturbs synaptic vesicle cycling. **J Clin Invest** 125: 1670–1678, 2015. doi: 10.1172/JCI79765.

416. Valente P, Castroflorio E, Rossi P, Fadda M, Sterlini B, Cervigni RI, et al. PRRT2 Is a Key Component of the Ca(2+)-Dependent Neurotransmitter Release Machinery. **Cell Rep** 15: 117–131, 2016. doi: 10.1016/j.celrep.2016.03.005.

417. Fruscione F, Valente P, Sterlini B, Romei A, Baldassari S, Fadda M, et al. PRRT2 controls neuronal excitability by negatively modulating Na+ channel 1.2/1.6 activity. **Brain** 141: 1000–1016, 2018. doi: 10.1093/brain/awy051.

418. Ferrante D, Sterlini B, Prestigio C, Marte A, Corradi A, Onofri F, et al. PRRT2 modulates presynaptic Ca2+ influx by interacting with P/Q-type channels. **Cell Rep** 35: 109248, 2021.

doi: 10.1016/j.celrep.2021.109248.

419. Ciruelas K, Marcotulli D, Bajjalieh SM. Synaptic vesicle protein 2: A multi-faceted regulator of secretion. **Semin Cell Dev Biol** 95: 130–141, 2019. doi: 10.1016/j.semcdb.2019.02.003.

420. Harper CB, Small C, Davenport EC, Low DW, Smillie KJ, Martínez-Mármol R, et al. An Epilepsy-Associated SV2A Mutation Disrupts Synaptotagmin-1 Expression and Activity-Dependent Trafficking. **J Neurosci** 40: 4586–4595, 2020. doi: 10.1523/JNEUROSCI.0210-20.2020.

421. Rizo J, Südhof TC. The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices--guilty as charged? **Annu Rev Cell Dev Biol** 28: 279–308, 2012. doi: 10.1146/annurev-cellbio-101011-155818.

422. Lai Y, Choi UB, Leitz J, Rhee HJ, Lee C, Altas B, et al. Molecular Mechanisms of Synaptic Vesicle Priming by Munc13 and Munc18. **Neuron** 95: 591--607.e10, 2017. doi: 10.1016/j.neuron.2017.07.004.

423. Engel AG, Selcen D, Shen XM, Milone M, Harper CM. Loss of MUNC13-1 function causes microcephaly, cortical hyperexcitability, and fatal myasthenia. **Neurol Genet** 2: 1–6, 2016. doi: 10.1212/NXG.0000000000000105.

424. Lanoue V, Chai YJ, Brouillet JZ, Weckhuysen S, Palmer EE, Collins BM, et al. STXBP1 encephalopathy: Connecting neurodevelopmental disorders with $α$-synucleinopathies? **Neurology** 93: 114–123, 2019. doi: 10.1212/WNL.0000000000007786.

425. Rost BR, Schneider F, Grauel MK, Wozny C, Bentz C, Blessing A, et al. Optogenetic acidification of synaptic vesicles and lysosomes. **Nat Neurosci** 18: 1845–1852, 2015. doi: 10.1038/nn.4161.

426. Bodzęta A, Kahms M, Klingauf J. The Presynaptic v-ATPase Reversibly Disassembles and Thereby Modulates Exocytosis but Is Not Part of the Fusion Machinery. **Cell Rep** 20: 1348–

1359, 2017. doi: 10.1016/j.celrep.2017.07.040.

427. Gowrisankaran S, Milosevic I. Regulation of synaptic vesicle acidification at the neuronal synapse. **IUBMB Life** 72: 568–576, 2020. doi: 10.1002/iub.2235.

428. Jaskolka MC, Winkley SR, Kane PM. RAVE and Rabconnectin-3 Complexes as Signal Dependent Regulators of Organelle Acidification. **Front Cell Dev Biol** 9: 698190, 2021. doi: 10.3389/fcell.2021.698190.

429. Fassio A, Esposito A, Kato M, Saitsu H, Mei D, Marini C, et al. De novo mutations of the ATP6V1A gene cause developmental encephalopathy with epilepsy. **Brain** 141: 1703–1718, 2018. doi: 10.1093/brain/awy092.

430. Aoto K, Kato M, Akita T, Nakashima M, Mutoh H, Akasaka N, et al. ATP6V0A1 encoding the a1-subunit of the V0 domain of vacuolar H+-ATPases is essential for brain development in humans and mice. **Nat Commun** 12: 2107, 2021. doi: 10.1038/s41467-021-22389-5.

431. Esposito A, Falace A, Wagner M, Gal M, Mei D, Conti V, et al. Biallelic DMXL2 mutations impair autophagy and cause Ohtahara syndrome with progressive course. **Brain** 142: 3876–3891, 2019. doi: 10.1093/brain/awz326.

432. Helbig I, Lopez-Hernandez T, Shor O, Galer P, Ganesan S, Pendziwiat M, et al. A Recurrent Missense Variant in AP2M1 Impairs Clathrin-Mediated Endocytosis and Causes Developmental and Epileptic Encephalopathy. **Am J Hum Genet** 104: 1060–1072, 2019. doi: 10.1016/j.ajhg.2019.04.001.

433. Gordon SL, Leube RE, Cousin MA. Synaptophysin is required for synaptobrevin retrieval during synaptic vesicle endocytosis. **J Neurosci** 31: 14032–14036, 2011. doi: 10.1523/JNEUROSCI.3162-11.2011.

434. Gordon SL, Cousin MA. X-linked intellectual disability-associated mutations in synaptophysin disrupt synaptobrevin II retrieval. **J Neurosci** 33: 13695–13700, 2013. doi:

10.1523/JNEUROSCI.0636-13.2013.

435. Harper CB, Mancini GMS, van Slegtenhorst M, Cousin MA. Altered synaptobrevin-II trafficking in neurons expressing a synaptophysin mutation associated with a severe neurodevelopmental disorder. **Neurobiol Dis** 108: 298–306, 2017. doi: 10.1016/j.nbd.2017.08.021.

436. von Spiczak S, Helbig KL, Shinde DN, Huether R, Pendziwiat M, Lourenço C, et al. DNM1 encephalopathy: A new disease of vesicle fission. **Neurology** 89: 385–394, 2017. doi: 10.1212/WNL.0000000000004152.

437. Morlot S, Roux A. Mechanics of dynamin-mediated membrane fission. **Annu Rev Biophys** 42: 629–649, 2013. doi: 10.1146/annurev-biophys-050511-102247.

438. Robinson MS. Forty Years of Clathrin-coated Vesicles. **Traffic** 16: 1210–1238, 2015. doi: 10.1111/tra.12335.

439. DeMari J, Mroske C, Tang S, Nimeh J, Miller R, Lebel RR. CLTC as a clinically novel gene associated with multiple malformations and developmental delay. **Am J Med Genet A** 170A: 958–966, 2016. doi: 10.1002/ajmg.a.37506.

440. Balestrini S, Milh M, Castiglioni C, Lüthy K, Finelli MJ, Verstreken P, et al. TBC1D24 genotype–phenotype correlation. **Neurology** 87: 77–85, 2016. doi: 10.1212/WNL.0000000000002807.

441. Falace A, Filipello F, La Padula V, Vanni N, Madia F, De Pietri Tonelli D, et al. TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. **Am J Hum Genet** 87: 365–370, 2010. doi: 10.1016/j.ajhg.2010.07.020.

442. Falace A, Buhler E, Fadda M, Watrin F, Lippiello P, Pallesi-Pocachard E, et al. TBC1D24 regulates neuronal migration and maturation through modulation of the ARF6-dependent pathway. **Proc Natl Acad Sci U S A** 111: 2337–2342, 2014. doi: 10.1073/pnas.1316294111.

443. Finelli MJ, Aprile D, Castroflorio E, Jeans A, Moschetta M, Chessum L, et al. The epilepsy-associated protein TBC1D24 is required for normal development, survival and vesicle trafficking in mammalian neurons. **Hum Mol Genet** 28: 584–597, 2019. doi: 10.1093/hmg/ddy370.

444. Aprile D, Fruscione F, Baldassari S, Fadda M, Ferrante D, Falace A, et al. TBC1D24 regulates axonal outgrowth and membrane trafficking at the growth cone in rodent and human neurons. **Cell Death Differ** 26: 2464–2478, 2019. doi: 10.1038/s41418-019-0313-x.

445. Fornasiero EF, Bonanomi D, Benfenati F, Valtorta F. The role of synapsins in neuronal development. **Cell Mol life Sci C** 67: 1383–1396, 2010. doi: 10.1007/s00018-009-0227-8.

446. Cesca F, Baldelli P, Valtorta F, Benfenati F. The synapsins: key actors of synapse function and plasticity. **Prog Neurobiol** 91: 313–348, 2010. doi: 10.1016/j.pneurobio.2010.04.006.

447. Fassio A, Raimondi A, Lignani G, Benfenati F, Baldelli P. Synapsins: from synapse to network hyperexcitability and epilepsy. **Semin Cell Dev Biol** 22: 408–415, 2011. doi: 10.1016/j.semcdb.2011.07.005.

448. Longhena F, Faustini G, Brembati V, Pizzi M, Benfenati F, Bellucci A. An updated reappraisal of synapsins: structure, function and role in neurological and psychiatric disorders. **Neurosci Biobehav Rev** 130: 33–60, 2021. doi: 10.1016/j.neubiorev.2021.08.011.

449. Greco B, Managò F, Tucci V, Kao H-T, Valtorta F, Benfenati F. Autism-related behavioral abnormalities in synapsin knockout mice. **Behav Brain Res** 251: 65–74, 2013. doi: 10.1016/j.bbr.2012.12.015.

450. Michetti C, Caruso A, Pagani M, Sabbioni M, Medrihan L, David G, et al. The Knockout of Synapsin II in Mice Impairs Social Behavior and Functional Connectivity Generating an ASD-like Phenotype. **Cereb Cortex** 27: 5014–5023, 2017. doi: 10.1093/cercor/bhx207.

451. Fassio A, Patry L, Congia S, Onofri F, Piton A, Gauthier J, et al. SYN1 loss-of-function

mutations in autism and partial epilepsy cause impaired synaptic function. **Hum Mol Genet** 20: 2297–2307, 2011. doi: 10.1093/hmg/ddr122.

452. Giannandrea M, Guarnieri FC, Gehring NH, Monzani E, Benfenati F, Kulozik AE, et al. Nonsense-mediated mRNA decay and loss-of-function of the protein underlie the X-linked epilepsy associated with the W356× mutation in synapsin I. **PLoS One** 8: e67724, 2013. doi: 10.1371/journal.pone.0067724.

453. Corradi A, Fadda M, Piton A, Patry L, Marte A, Rossi P, et al. SYN2 is an autism predisposing gene: loss-of-function mutations alter synaptic vesicle cycling and axon outgrowth. **Hum Mol Genet** 23: 90–103, 2014. doi: 10.1093/hmg/ddt401.

454. Nguyen DK, Rouleau I, Sénéchal G, Ansaldo AI, Gravel M, Benfenati F, et al. X-linked focal epilepsy with reflex bathing seizures: Characterization of a distinct epileptic syndrome. **Epilepsia** 56: 1098–1108, 2015. doi: 10.1111/epi.13042.

455. Guarnieri FC, Pozzi D, Raimondi A, Fesce R, Valente MM, Delvecchio VS, et al. A novel SYN1 missense mutation in non-syndromic X-linked intellectual disability affects synaptic vesicle life cycle, clustering and mobility. **Hum Mol Genet** 26: 4699–4714, 2017. doi: 10.1093/hmg/ddx352.

456. Accogli A, Wiegand G, Scala M, Cerminara C, Iacomino M, Riva A, et al. Clinical and Genetic Features in Patients With Reflex Bathing Epilepsy. **Neurology** 97: e577--e586, 2021. doi: 10.1212/WNL.0000000000012298.

457. Rogawski MA. A New SV2A Ligand for Epilepsy. **Cell** 167: 587, 2016. doi: 10.1016/j.cell.2016.09.057.

458. Benke TA, Park K, Krey I, Camp CR, Song R, Ramsey AJ, et al. Clinical and therapeutic significance of genetic variation in the GRIN gene family encoding NMDARs. **Neuropharmacology** 199: 108805, 2021. doi: 10.1016/j.neuropharm.2021.108805.

459. Lemke JR, Lal D, Reinthaler EM, Steiner I, Nothnagel M, Alber M, et al. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. **Nat Genet** 45: 1067–1072, 2013. doi: 10.1038/ng.2728.

460. Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. **Nat Genet** 45: 1061–1066, 2013. doi: 10.1038/ng.2726.

461. Platzer K, Yuan H, Schütz H, Winschel A, Chen W, Hu C, et al. GRIN2B encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects. **J Med Genet** 54: 460–470, 2017. doi: 10.1136/jmedgenet-2016-104509.

462. Bertocchi I, Eltokhi A, Rozov A, Chi VN, Jensen V, Bus T, et al. Voltage-independent GluN2A-type NMDA receptor Ca2+ signaling promotes audiogenic seizures, attentional and cognitive deficits in mice. **Commun Biol** 4: 59, 2021. doi: 10.1038/s42003-020-01538-4.

463. Shin W, Kim K, Serraz B, Cho YS, Kim D, Kang M, et al. Early correction of synaptic long-term depression improves abnormal anxiety-like behavior in adult GluN2B-C456Y-mutant mice. **PLOS Biol** 18: e3000717, 2020. doi: 10.1371/journal.pbio.3000717.

464. Amador A, Bostick CD, Olson H, Peters J, Camp CR, Krizay D, et al. Modelling and treating GRIN2A developmental and epileptic encephalopathy in mice. **Brain** 143: 2039–2057, 2020. doi: 10.1093/brain/awaa147.

465. Mayer ML. Glutamate receptor ion channels. **Curr Opin Neurobiol** 15: 282–288, 2005. doi: 10.1016/j.conb.2005.05.004.

466. Greger IH, Ziff EB, Penn AC. Molecular determinants of AMPA receptor subunit assembly. **Trends Neurosci** 30: 407–416, 2007. doi: 10.1016/j.tins.2007.06.005.

467. Salpietro V, Dixon CL, Guo H, Bello OD, Vandrovcova J, Efthymiou S, et al. AMPA receptor

GluA2 subunit defects are a cause of neurodevelopmental disorders. **Nat Commun** 10: 3094, 2019. doi: 10.1038/s41467-019-10910-w.

468. Jia Z, Agopyan N, Miu P, Xiong Z, Henderson J, Gerlai R, et al. Enhanced LTP in Mice Deficient in the AMPA Receptor GluR2. **Neuron** 17: 945–956, 1996. doi: 10.1016/S0896-6273(00)80225-1.

469. Wu Y, Arai AC, Rumbaugh G, Srivastava AK, Turner G, Hayashi T, et al. Mutations in ionotropic AMPA receptor 3 alter channel properties and are associated with moderate cognitive impairment in humans. **Proc Natl Acad Sci** 104: 18163–18168, 2007. doi: 10.1073/pnas.0708699104.

470. Trivisano M, Santarone ME, Micalizzi A, Ferretti A, Dentici ML, Novelli A, et al. GRIA3 missense mutation is cause of an x-linked developmental and epileptic encephalopathy. **Seizure** 82: 1–6, 2020. doi: 10.1016/j.seizure.2020.08.032.

471. Martin S, Chamberlin A, Shinde DN, Hempel M, Strom TM, Schreiber A, et al. De Novo Variants in GRIA4 Lead to Intellectual Disability with or without Seizures and Gait Abnormalities. **Am J Hum Genet** 101: 1013–1020, 2017. doi: 10.1016/j.ajhg.2017.11.004.

472. Maljevic S, Møller RS, Reid CA, Pérez-Palma E, Lal D, May P, et al. Spectrum of GABAA receptor variants in epilepsy. **Curr Opin Neurol** 32: 183–190, 2019. doi: 10.1097/WCO.0000000000000657.

473. Butler KM, Moody OA, Schuler E, Coryell J, Alexander JJ, Jenkins A, et al. De novo variants in GABRA2 and GABRA5 alter receptor function and contribute to early-onset epilepsy. **Brain** 141: 2392–2405, 2018. doi: 10.1093/brain/awy171.

474. Maljevic S, Keren B, Aung YH, Forster IC, Mignot C, Buratti J, et al. Novel GABRA2 variants in epileptic encephalopathy and intellectual disability with seizures. **Brain** 142: e15--e15, 2019. doi: 10.1093/brain/awz079.

475. Hernandez CC, XiangWei W, Hu N, Shen D, Shen W, Lagrange AH, et al. Altered inhibitory synapses in de novo GABRA5 and GABRA1 mutations associated with early onset epileptic encephalopathies. **Brain** 142: 1938–1954, 2019. doi: 10.1093/brain/awz123.

476. Carvill GL, Weckhuysen S, McMahon JM, Hartmann C, Moller RS, Hjalgrim H, et al. GABRA1 and STXBP1: Novel genetic causes of Dravet syndrome. **Neurology** 82: 1245–1253, 2014. doi: 10.1212/WNL.0000000000000291.

477. Janve VS, Hernandez CC, Verdier KM, Hu N, Macdonald RL. Epileptic encephalopathy de novo GABRB mutations impair $γ$-aminobutyric acid type A receptor function. **Ann Neurol** 79: 806–825, 2016. doi: 10.1002/ana.24631.

478. Ishii A, Kang J-Q, Schornak CC, Hernandez CC, Shen W, Watkins JC, et al. A de novo missense mutation of GABRB2 causes early myoclonic encephalopathy. **J Med Genet** 54: 202–211, 2017. doi: 10.1136/jmedgenet-2016-104083.

479. Shen D, Hernandez CC, Shen W, Hu N, Poduri A, Shiedley B, et al. De novo GABRG2 mutations associated with epileptic encephalopathies. **Brain** 140: 49–67, 2017. doi: 10.1093/brain/aww272.

480. Ahring PK, Liao VWY, Gardella E, Johannesen KM, Krey I, Selmer KK, et al. Gain-of-function variants in GABRD reveal a novel pathway for neurodevelopmental disorders and epilepsy. **Brain** Oct 11;awab391, 2021 doi: 10.1093/brain/awab391.

481. Mermer F, Poliquin S, Rigsby K, Rastogi A, Shen W, Romero-Morales A, et al. Common molecular mechanisms of SLC6A1 variant-mediated neurodevelopmental disorders in astrocytes and neurons. **Brain** 144: 2499–2512, 2021. doi: 10.1093/brain/awab207.

482. Arain FM, Boyd KL, Gallagher MJ. Decreased viability and absence-like epilepsy in mice lacking or deficient in the GABAA receptor $α$1 subunit. **Epilepsia** 53: e161--e165, 2012. doi: 10.1111/j.1528-1167.2012.03596.x.

483. Yeung RK, Xiang Z-H, Tsang S-Y, Li R, Ho TYC, Li Q, et al. Gabrb2-knockout mice displayed schizophrenia-like and comorbid phenotypes with interneuron–astrocyte–microglia dysregulation. **Transl Psychiatry** 8: 128, 2018. doi: 10.1038/s41398-018-0176-9.

484. Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, et al. Mice devoid of -aminobutyrate type A receptor 3 subunit have epilepsy, cleft palate, and hypersensitive behavior. **Proc Natl Acad Sci** 94: 4143–4148, 1997. doi: 10.1073/pnas.94.8.4143.

485. DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, et al. Mice Lacking the $β$ 3 Subunit of the GABA A Receptor Have the Epilepsy Phenotype and Many of the Behavioral Characteristics of Angelman Syndrome. **J Neurosci** 18: 8505–8514, 1998. doi: 10.1523/JNEUROSCI.18-20-08505.1998.

486. Schofield CM, Kleiman-Weiner M, Rudolph U, Huguenard JR. A gain in GABAA receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures. **Proc Natl Acad Sci** 106: 7630–7635, 2009. doi: 10.1073/pnas.0811326106.

487. Warner TA, Shen W, Huang X, Liu Z, Macdonald RL, Kang J-Q. Differential molecular and behavioural alterations in mouse models of GABRG2 haploinsufficiency versus dominant negative mutations associated with human epilepsy. **Hum Mol Genet** 25: 3192–3207, 2016. doi: 10.1093/hmg/ddw168.

488. Yoo Y, Jung J, Lee Y-N, Lee Y, Cho H, Na E, et al. GABBR2 mutations determine phenotype in rett syndrome and epileptic encephalopathy. **Ann Neurol** 82: 466–478, 2017. doi: 10.1002/ana.25032.

489. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J. Cation-chloride cotransporters in neuronal development, plasticity and disease. **Nat Rev Neurosci** 15: 637–654, 2014. doi: 10.1038/nrn3819.

490. Di Cristo G, Awad PN, Hamidi S, Avoli M. KCC2, epileptiform synchronization, and epileptic disorders. **Prog Neurobiol** 162: 1–16, 2018. doi: 10.1016/j.pneurobio.2017.11.002.

491. Kahle KT, Khanna AR, Duan J, Staley KJ, Delpire E, Poduri A. The KCC2 Cotransporter and Human Epilepsy. **Neurosci** 22: 555–562, 2016. doi: 10.1177/1073858416645087.

492. Puskarjov M, Seja P, Heron SE, Williams TC, Ahmad F, Iona X, et al. A variant of KCC2 from patients with febrile seizures impairs neuronal Cl- extrusion and dendritic spine formation. **EMBO Rep** 15: 723–729, 2014. doi: 10.1002/embr.201438749.

493. Jentsch TJ, Pusch M. CLC Chloride Channels and Transporters: Structure, Function, Physiology, and Disease. **Physiol Rev** 98: 1493–1590, 2018. doi: 10.1152/physrev.00047.2017.

494. He H, Guzman RE, Cao D, Sierra-Marquez J, Yin F, Fahlke C, et al. The molecular and phenotypic spectrum of CLCN4 -related epilepsy. **Epilepsia** 62: 1401–1415, 2021. doi: 10.1111/epi.16906.

495. Palmer EE, Stuhlmann T, Weinert S, Haan E, Van Esch H, Holvoet M, et al. De novo and inherited mutations in the X-linked gene CLCN4 are associated with syndromic intellectual disability and behavior and seizure disorders in males and females. **Mol Psychiatry** 23: 222–230, 2018. doi: 10.1038/mp.2016.135.

496. Stergachis AB, Pujol-Giménez J, Gyimesi G, Fuster D, Albano G, Troxler M, et al. Recurrent SLC1A2 variants cause epilepsy via a dominant negative mechanism. **Ann Neurol** 85: 921–926, 2019. doi: 10.1002/ana.25477.

497. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, et al. Epilepsy and Exacerbation of Brain Injury in Mice Lacking the Glutamate Transporter GLT-1. **Science** 276: 1699–1702, 1997. doi: 10.1126/science.276.5319.1699.

498. Goodspeed K, Pérez-Palma E, Iqbal S, Cooper D, Scimemi A, Johannesen KM, et al. Current

knowledge of SLC6A1-related neurodevelopmental disorders. **Brain Commun** 2: fcaa170, 2020. doi: 10.1093/braincomms/fcaa170. Oct 13;2(2):fcaa170.

499. Bröer S, Gether U. The solute carrier 6 family of transporters. **Br J Pharmacol** 167: 256–278, 2012. doi: 10.1111/j.1476-5381.2012.01975.x.

500. Carvill GL, Heavin SB, Yendle SC, McMahon JM, O'Roak BJ, Cook J, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. **Nat Genet** 45: 825–830, 2013. doi: 10.1038/ng.2646.

501. Menuz K, Nicoll RA. Loss of inhibitory neuron AMPA receptors contributes to ataxia and epilepsy in stargazer mice. **J Neurosci** 28: 10599–10603, 2008. doi: 10.1523/JNEUROSCI.2732-08.2008.

502. Harrison V, Connell L, Hayesmoore J, McParland J, Pike MG, Blair E. Compound heterozygous deletion of NRXN1 causing severe developmental delay with early onset epilepsy in two sisters. **Am J Med Genet A** 155A: 2826–2831, 2011. doi: 10.1002/ajmg.a.34255.

503. Dinopoulos A, Stefanou M-I, Attilakos A, Tsirouda M, Papaevangelou V. A case of startle epilepsy associated with IL1RAPL1 gene deletion. **Pediatr Neurol** 51: 271–274, 2014. doi: 10.1016/j.pediatrneurol.2014.04.011.

504. Rodenas-Cuadrado P, Pietrafusa N, Francavilla T, La Neve A, Striano P, Vernes SC. Characterisation of CASPR2 deficiency disorder--a syndrome involving autism, epilepsy and language impairment. **BMC Med Genet** 17: 8, 2016. doi: 10.1186/s12881-016-0272-8.

505. Kalachikov S, Evgrafov O, Ross B, Winawer M, Barker-Cummings C, Martinelli Boneschi F, et al. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. **Nat Genet** 30: 335–341, 2002. doi: 10.1038/ng832.

506. Lovero KL, Fukata Y, Granger AJ, Fukata M, Nicoll RA. The LGI1-ADAM22 protein complex

directs synapse maturation through regulation of PSD-95 function. **Proc Natl Acad Sci U S A** 112: E4129--4137, 2015. doi: 10.1073/pnas.1511910112.

507. Schirwani S, McConnell V, Willoughby J, Study DDD, Balasubramanian M. Exploring the association between SRPX2 variants and neurodevelopment: How causal is it? **Gene** 685: 50–54, 2019. doi: 10.1016/j.gene.2018.10.067.

508. Dazzo E, Fanciulli M, Serioli E, Minervini G, Pulitano P, Binelli S, et al. Heterozygous reelin mutations cause autosomal-dominant lateral temporal epilepsy. **Am J Hum Genet** 96: 992–1000, 2015. doi: 10.1016/j.ajhg.2015.04.020.

509. Michelucci R, Pulitano P, Di Bonaventura C, Binelli S, Luisi C, Pasini E, et al. The clinical phenotype of autosomal dominant lateral temporal lobe epilepsy related to reelin mutations. **Epilepsy Behav** 68: 103–107, 2017. doi: 10.1016/j.yebeh.2016.12.003.

510. Dazzo E, Nobile C. Epilepsy-causing Reelin mutations result in impaired secretion and intracellular degradation of mutant proteins. **Hum Mol Genet** 31 :665-673, 2021. doi: 10.1093/hmg/ddab271.

511. Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. **Nat Rev Mol Cell Biol** 19: 349–364, 2018. doi: 10.1038/s41580-018-0003-4.

512. Fassio A, Falace A, Esposito A, Aprile D, Guerrini R, Benfenati F. Emerging Role of the Autophagy/Lysosomal Degradative Pathway in Neurodevelopmental Disorders With Epilepsy. **Front Cell Neurosci** 14: 39, 2020. doi: 10.3389/fncel.2020.00039.

513. Rocchi A, Carminati E, De Fusco A, Kowalska JA, Floss T, Benfenati F. REST/NRSF deficiency impairs autophagy and leads to cellular senescence in neurons. **Aging Cell** 20: e13471, 2021. doi: 10.1111/acel.13471.

514. Switon K, Kotulska K, Janusz-Kaminska A, Zmorzynska J, Jaworski J. Molecular neurobiology of mTOR. **Neuroscience** 341: 112–153, 2017. doi: 10.1016/j.neuroscience.2016.11.017.

515. Nguyen LH, Bordey A. Corrigendum: Convergent and Divergent Mechanisms of Epileptogenesis in mTORopathies. **Front Neuroanat** 15: 715363, 2021. doi: 10.3389/fnana.2021.715363.

516. Baulac S. mTOR signaling pathway genes in focal epilepsies. **Prog Brain Res** 226: 61–79, 2016. doi: 10.1016/bs.pbr.2016.04.013.

517. Moloney PB, Cavalleri GL, Delanty N. Epilepsy in the mTORopathies: opportunities for precision medicine. **Brain Commun** 3: fcab222, 2021. doi: 10.1093/braincomms/fcab222.

518. Lasarge CL, Danzer SC. Mechanisms regulating neuronal excitability and seizure development following mTOR pathway hyperactivation. **Front Mol Neurosci** 7: 18, 2014. doi: 10.3389/fnmol.2014.00018.

519. LaSarge CL, Pun RYK, Gu Z, Riccetti MR, Namboodiri D V, Tiwari D, et al. mTOR-driven neural circuit changes initiate an epileptogenic cascade. **Prog Neurobiol** 200: 101974, 2021. doi: 10.1016/j.pneurobio.2020.101974.

520. Ribierre T, Deleuze C, Bacq A, Baldassari S, Marsan E, Chipaux M, et al. Second-hit mosaic mutation in mTORC1 repressor DEPDC5 causes focal cortical dysplasia-associated epilepsy. **J Clin Invest** 128: 2452–2458, 2018. doi: 10.1172/JCI99384.

521. De Fusco A, Cerullo MS, Marte A, Michetti C, Romei A, Castroflorio E, et al. Acute knockdown of Depdc5 leads to synaptic defects in mTOR-related epileptogenesis. **Neurobiol Dis** 139: 104822, 2020. doi: 10.1016/j.nbd.2020.104822.

522. Menzies FM, Fleming A, Caricasole A, Bento CF, Andrews SP, Ashkenazi A, et al. Autophagy and Neurodegeneration: Pathogenic Mechanisms and Therapeutic Opportunities. **Neuron** 93: 1015–1034, 2017. doi: 10.1016/j.neuron.2017.01.022.

523. Byrne S, Jansen L, U-King-Im J-M, Siddiqui A, Lidov HGW, Bodi I, et al. EPG5-related Vici syndrome: a paradigm of neurodevelopmental disorders with defective autophagy. **Brain**

139: 765–781, 2016. doi: 10.1093/brain/awv393.

524. Thomas AC, Williams H, Setó-Salvia N, Bacchelli C, Jenkins D, O'Sullivan M, et al. Mutations in SNX14 cause a distinctive autosomal-recessive cerebellar ataxia and intellectual disability syndrome. **Am J Hum Genet** 95: 611–621, 2014. doi: 10.1016/j.ajhg.2014.10.007.

525. Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. **Nat Rev Mol Cell Biol** 8: 917–929, 2007. doi: 10.1038/nrm2272.

526. Cotter K, Stransky L, McGuire C, Forgac M. Recent Insights into the Structure, Regulation, and Function of the V-ATPases. **Trends Biochem Sci** 40: 611–622, 2015. doi: 10.1016/j.tibs.2015.08.005.

527. Morel N, Poëa-Guyon S. The membrane domain of vacuolar H(+)ATPase: a crucial player in neurotransmitter exocytotic release. **Cell Mol life Sci C** 72: 2561–2573, 2015. doi: 10.1007/s00018-015-1886-2.

528. Van Damme T, Gardeitchik T, Mohamed M, Guerrero-Castillo S, Freisinger P, Guillemyn B, et al. Mutations in ATP6V1E1 or ATP6V1A Cause Autosomal-Recessive Cutis Laxa. **Am J Hum Genet** 100: 216–227, 2017. doi: 10.1016/j.ajhg.2016.12.010.

529. Wang M, Li A, Sekiya M, Beckmann ND, Quan X, Schrode N, et al. Transformative Network Modeling of Multi-omics Data Reveals Detailed Circuits, Key Regulators, and Potential Therapeutics for Alzheimer's Disease. **Neuron** 109: 257--272.e14, 2021. doi: 10.1016/j.neuron.2020.11.002.

530. Hirose T, Cabrera-Socorro A, Chitayat D, Lemonnier T, Féraud O, Cifuentes-Diaz C, et al. ATP6AP2 variant impairs CNS development and neuronal survival to cause fulminant neurodegeneration. **J Clin Invest** 129: 2145–2162, 2019. doi: 10.1172/JCI79990.

531. Nagano F, Kawabe H, Nakanishi H, Shinohara M, Deguchi-Tawarada M, Takeuchi M, et al. Rabconnectin-3, a novel protein that binds both GDP/GTP exchange protein and GTPase-

activating protein for Rab3 small G protein family. **J Biol Chem** 277: 9629–9632, 2002. doi: 10.1074/jbc.C100730200.

532. Kawabe H, Sakisaka T, Yasumi M, Shingai T, Izumi G, Nagano F, et al. A novel rabconnectin-3-binding protein that directly binds a GDP/GTP exchange protein for Rab3A small G protein implicated in Ca(2+)-dependent exocytosis of neurotransmitter. **Genes Cells** 8: 537–546, 2003. doi: 10.1046/j.1365-2443.2003.00655.x.

533. Tata B, Huijbregts L, Jacquier S, Csaba Z, Genin E, Meyer V, et al. Haploinsufficiency of Dmxl2, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse. **PLoS Biol** 12: e1001952, 2014. doi: 10.1371/journal.pbio.1001952.

534. Kannan M, Bayam E, Wagner C, Rinaldi B, Kretz PF, Tilly P, et al. WD40-repeat 47, a microtubule-associated protein, is essential for brain development and autophagy. **Proc Natl Acad Sci U S A** 114: E9308--E9317, 2017. doi: 10.1073/pnas.1713625114.

535. Ebrahimi-Fakhari D, Saffari A, Wahlster L, Lu J, Byrne S, Hoffmann GF, et al. Congenital disorders of autophagy: an emerging novel class of inborn errors of neuro-metabolism. **Brain** 139: 317–337, 2016. doi: 10.1093/brain/awv371.

536. Löscher W. Single-Target Versus Multi-Target Drugs Versus Combinations of Drugs With Multiple Targets: Preclinical and Clinical Evidence for the Treatment or Prevention of Epilepsy. **Front Pharmacol** 12: 730257, 2021. doi: 10.3389/fphar.2021.730257.

537. Chachua T, Yum M-S, Velíšková J, Velíšek L. Validation of the rat model of cryptogenic infantile spasms. **Epilepsia** 52: 1666–1677, 2011. doi: 10.1111/j.1528-1167.2011.03220.x.

538. Chern C-R, Chern C-J, Velíšková J, Velíšek L. AQB-565 shows promise in preclinical testing in the model of epileptic spasms during infancy: Head-to-head comparison with ACTH. **Epilepsy Res** 152: 31–34, 2019. doi: 10.1016/j.eplepsyres.2019.03.004.

539. Blichowski M, Shephard A, Armstrong J, Shen L, Cortez MA, Eubanks JH, et al. The GIRK2

3512 subunit is involved in IS-like seizures induced by GABA(B) receptor agonists. **Epilepsia** 56:
3513 1081–1087, 2015. doi: 10.1111/epi.13034.
3514 540. Yum M-S, Lee M, Ko T-S, Velíšek L. A potential effect of ganaxolone in an animal model of
3515 infantile spasms. **Epilepsy Res** 108: 1492–1500, 2014. doi:
3516 10.1016/j.eplepsyres.2014.08.015.
3517 541. Chern C-R, Chern C-J, Velíšková J, Velíšek L. ACTON PROLONGATUM® suppresses spasms
3518 head to head with Acthar® Gel in the model of infantile spasms. **Epilepsy Behav** 105:
3519 106950, 2020. doi: 10.1016/j.yebeh.2020.106950.
3520 542. Chachua T, Di Grazia P, Chern C-R, Johnkutty M, Hellman B, Lau HA, et al. Estradiol does not
3521 affect spasms in the betamethasone-NMDA rat model of infantile spasms. **Epilepsia** 57:
3522 1326–1336, 2016. doi: 10.1111/epi.13434.
3523 543. Yum M-S, Lee M, Woo D-C, Kim DW, Ko T-S, Velíšek L. $β$-Hydroxybutyrate attenuates
3524 NMDA-induced spasms in rats with evidence of neuronal stabilization on MR spectroscopy.
3525 **Epilepsy Res** 117: 125–132, 2015. doi: 10.1016/j.eplepsyres.2015.08.005.
3526 544. Janicot R, Stafstrom CE, Shao L-R. 2-Deoxyglucose terminates pilocarpine-induced status
3527 epilepticus in neonatal rats. **Epilepsia** 61: 1528–1537, 2020. doi: 10.1111/epi.16583.
3528 545. Jackson MR, Lee K, Mattiske T, Jaehne EJ, Ozturk E, Baune BT, et al. Extensive phenotyping
3529 of two ARX polyalanine expansion mutation mouse models that span clinical spectrum of
3530 intellectual disability and epilepsy. **Neurobiol Dis** 105: 245–256, 2017. doi:
3531 10.1016/j.nbd.2017.05.012.
3532 546. Le JT, Frost JD, Swann JW. Acthar®Gel (repository corticotropin injection) dose-response
3533 relationships in an animal model of epileptic spasms. **Epilepsy Behav** 116: 107786, 2021.
3534 doi: 10.1016/j.yebeh.2021.107786.
3535 547. Doumlele K, Conway E, Hedlund J, Tolete P, Devinsky O. A case report on the efficacy of

vigabatrin analogue (1S, 3S)-3-amino-4-difluoromethylenyl-1-cyclopentanoic acid (CPP-115) in a patient with infantile spasms. **Epilepsy Behav Case Reports** 6: 67–69, 2016. doi: 10.1016/j.ebcr.2016.08.002.

548. Samueli S, Dressler A, Gröppel G, Scholl T, Feucht M. Everolimus in infants with tuberous sclerosis complex–related West syndrome: First results from a single-center prospective observational study. **Epilepsia** 59: e142–e146, 2018. doi: 10.1111/epi.14529.

549. Hawkins NA, Jurado M, Thaxton TT, Duarte SE, Barse L, Tatsukawa T, et al. Soticlestat, a novel cholesterol 24-hydroxylase inhibitor, reduces seizures and premature death in Dravet syndrome mice. **Epilepsia** 62: 2845–2857, 2021. doi: 10.1111/epi.17062.

550. Hawkins NA, Nomura T, Duarte S, Barse L, Williams RW, Homanics GE, et al. Gabra2 is a genetic modifier of Dravet syndrome in mice. **Mamm Genome** 32: 350–363, 2021. doi: 10.1007/s00335-021-09877-1.

551. Jancovski N, Baldwin T, Orford M, Li M, Jones GD, Burbano LE, et al. Protective effects of medium chain triglyceride diet in a mouse model of Dravet syndrome. Epilepsia 62: 3131-3142, 2021. doi: 10.1111/epi.17101.

552. Satpute Janve V, Anderson LL, Bahceci D, Hawkins NA, Kearney JA, Arnold JC. The Heat Sensing Trpv1 Receptor Is Not a Viable Anticonvulsant Drug Target in the Scn1a +/- Mouse Model of Dravet Syndrome. **Front Pharmacol** 12: 675128, 2021. doi: 10.3389/fphar.2021.675128.

553. Anderson LL, Ametovski A, Lin Luo J, Everett-Morgan D, McGregor IS, Banister SD, et al. Cannabichromene, Related Phytocannabinoids, and 5-Fluoro-cannabichromene Have Anticonvulsant Properties in a Mouse Model of Dravet Syndrome. **ACS Chem Neurosci** 12: 330–339, 2021. doi: 10.1021/acschemneuro.0c00677.

554. Anderson LL, Heblinski M, Absalom NL, Hawkins NA, Bowen MT, Benson MJ, et al.

Cannabigerolic acid, a major biosynthetic precursor molecule in cannabis, exhibits divergent effects on seizures in mouse models of epilepsy. **Br J Pharmacol** 178: 4826-4841, 2021.doi: 10.1111/bph.15661.

555. Crotts MS, Kim Y, Bravo E, Richerson GB, Teran FA. A ketogenic diet protects DBA/1 and Scn1aR1407X/+ mice against seizure-induced respiratory arrest independent of ketosis. **Epilepsy Behav** 124: 108334, 2021. doi: 10.1016/j.yebeh.2021.108334.

556. Mora-Jimenez L, Valencia M, Sanchez-Carpintero R, Tønnesen J, Fadila S, Rubinstein M, et al. Transfer of SCN1A to the brain of adolescent mouse model of Dravet syndrome improves epileptic, motor, and behavioral manifestations. **Mol Ther Nucleic Acids** 25: 585–602, 2021. doi: 10.1016/j.omtn.2021.08.003.

557. Hatini PG, Commons KG. A 5-HT1D -receptor agonist protects Dravet syndrome mice from seizure and early death. **Eur J Neurosci** 52: 4370–4374, 2020. doi: 10.1111/ejn.14776.

558. Yamagata T, Raveau M, Kobayashi K, Miyamoto H, Tatsukawa T, Ogiwara I, et al. CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons ameliorates epileptic and behavioral phenotypes of Dravet syndrome model mice. **Neurobiol Dis** 141: 104954, 2020. doi: 10.1016/j.nbd.2020.104954.

559. Sturgeon ML, Langton R, Sharma S, Cornell RA, Glykys J, Bassuk AG. The opioid antagonist naltrexone decreases seizure-like activity in genetic and chemically induced epilepsy models. **Epilepsia open** 6: 528–538, 2021. doi: 10.1002/epi4.12512.

560. Li J, Nelis M, Sourbron J, Copmans D, Lagae L, Cabooter D, et al. Efficacy of Fenfluramine and Norfenfluramine Enantiomers and Various Antiepileptic Drugs in a Zebrafish Model of Dravet Syndrome. **Neurochem Res** 46: 2249–2261, 2021. doi: 10.1007/s11064-021-03358-2.

561. Dinday MT, Baraban SC. Large-Scale Phenotype-Based Antiepileptic Drug Screening in a

Zebrafish Model of Dravet Syndrome. **Eneuro** 2: ENEURO.0068-15.2015, 2015. doi: 10.1523/ENEURO.0068-15.2015.

562. Griffin A, Hamling KR, Knupp K, Hong S, Lee LP, Baraban SC. Clemizole and modulators of serotonin signalling suppress seizures in Dravet syndrome. **Brain** 140: 669–683, 2017. doi: 10.1093/brain/aww342.

563. Banerji R, Huynh C, Figueroa F, Dinday MT, Baraban SC, Patel M. Enhancing glucose metabolism via gluconeogenesis is therapeutic in a zebrafish model of Dravet syndrome. **Brain Commun** 3: fcab004, 2021. doi: 10.1093/braincomms/fcab004.

564. Weuring WJ, Singh S, Volkers L, Rook MB, van ’t Slot RH, Bosma M, et al. NaV1.1 and NaV1.6 selective compounds reduce the behavior phenotype and epileptiform activity in a novel zebrafish model for Dravet Syndrome. **PLoS One** 15: e0219106, 2020. doi: 10.1371/journal.pone.0219106.

565. Cross JH, Galer BS, Gil-Nagel A, Devinsky O, Ceulemans B, Lagae L, et al. Impact of fenfluramine on the expected SUDEP mortality rates in patients with Dravet syndrome. **Seizure** 93: 154–159, 2021. doi: 10.1016/j.seizure.2021.10.024.

566. Sullivan J, Specchio N, Devinsky O, Auvin S, Perry MS, Strzelczyk A, et al. Fenfluramine significantly reduces day-to-day seizure burden by increasing number of seizure-free days and time between seizures in patients with Dravet syndrome: A time-to-event analysis. **Epilepsia** 63: 130–138, 2022. doi: 10.1111/epi.17106.

567. Ceulemans B, Boel M, Leyssens K, Van Rossem C, Neels P, Jorens PG, et al. Successful use of fenfluramine as an add-on treatment for Dravet syndrome. **Epilepsia** 53: 1131–1139, 2012. doi: 10.1111/j.1528-1167.2012.03495.x.

568. Schoonjans A, Paelinck BP, Marchau F, Gunning B, Gammaitoni A, Galer BS, et al. Low-dose fenfluramine significantly reduces seizure frequency in Dravet syndrome: a prospective

study of a new cohort of patients. **Eur J Neurol** 24: 309–314, 2017. doi: 10.1111/ene.13195.

569. Lagae L, Sullivan J, Knupp K, Laux L, Polster T, Nikanorova M, et al. Fenfluramine hydrochloride for the treatment of seizures in Dravet syndrome: a randomised, double-blind, placebo-controlled trial. **Lancet** 394: 2243–2254, 2019. doi: 10.1016/S0140-6736(19)32500-0.

570. Guerrini R, Balestrini S, Wirrell EC, Walker MC. Monogenic Epilepsies: Disease Mechanisms, Clinical Phenotypes, and Targeted Therapies. **Neurology** 97: 817–831, 2021. doi: 10.1212/WNL.0000000000012744.

571. Liu D, Zhu M, Zhang Y, Diao Y. Crossing the blood-brain barrier with AAV vectors. **Metab Brain Dis** 36: 45–52, 2021. doi: 10.1007/s11011-020-00630-2.

572. Turner TJ, Zourray C, Schorge S, Lignani G. Recent advances in gene therapy for neurodevelopmental disorders with epilepsy. **J Neurochem** 157: 229–262, 2021. doi: 10.1111/jnc.15168.

573. Morris G, O'Brien D, Henshall DC. Opportunities and challenges for microRNA-targeting therapeutics for epilepsy. **Trends Pharmacol Sci** 42: 605–616, 2021. doi: 10.1016/j.tips.2021.04.007.

574. Mooney C, Becker BA, Raoof R, Henshall DC. EpimiRBase: a comprehensive database of microRNA-epilepsy associations. **Bioinformatics** 32: 1436–1438, 2016. doi: 10.1093/bioinformatics/btw008.

575. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. **Cell** 157: 1262–1278, 2014. doi: 10.1016/j.cell.2014.05.010.

576. Redman M, King A, Watson C, King D. What is CRISPR/Cas9? **Arch Dis Child Educ Pract Ed** 101: 213–215, 2016. doi: 10.1136/archdischild-2016-310459.

577. Guerrini R. Epilepsy in children. **Lancet** 367: 499–524, 2006. doi: 10.1016/S0140-

6736(06)68182-8.

578. Shewmon DA, Erwin RJ. The effect of focal interictal spikes on perception and reaction time. II. Neuroanatomic specificity. **Electroencephalogr Clin Neurophysiol** 69: 338–352, 1988. doi: 10.1016/0013-4694(88)90005-3.

579. Shewmon DA, Erwin RJ. Focal spike-induced cerebral dysfunction is related to the after-coming slow wave. **Ann Neurol** 23: 131–137, 1988. doi: 10.1002/ana.410230205.

580. Brancati C, Barba C, Metitieri T, Melani F, Pellacani S, Viggiano MP, et al. Impaired object identification in idiopathic childhood occipital epilepsy. **Epilepsia** 53: 686–694, 2012. doi: 10.1111/j.1528-1167.2012.03410.x.

581. Chilosi AM, Brovedani P, Ferrari AR, Ziegler A-L, Guerrini R, Deonna T. Language Regression Associated With Autistic Regression and Electroencephalographic (EEG) Abnormalities. **J Child Neurol** 29: 855–859, 2014. doi: 10.1177/0883073813482767.

582. Gordon K, Bawden H, Camfield P, Mann S, Orlik P. Valproic Acid Treatment of Learning Disorder and Severely Epileptiform EEG Without Clinical Seizures. **J Child Neurol** 11: 41–43, 1996. doi: 10.1177/088307389601100110.

583. Guzzetta F, Frisone MF, Ricci D, Randò T, Guzzetta A. Development of Visual Attention in West Syndrome. **Epilepsia** 43: 757–763, 2002. doi: 10.1046/j.1528-1157.2002.34601.x.

584. Kábová R, Liptáková S, Slamberová R, Pometlová M, Velísek L. Age-specific N-methyl-D-aspartate-induced seizures: perspectives for the West syndrome model. **Epilepsia** 40: 1357–1369, 1999. doi: 10.1111/j.1528-1157.1999.tb02006.x.

585. Mares P, Velísek L. N-methyl-D-aspartate (NMDA)-induced seizures in developing rats. **Brain Res Dev Brain Res** 65: 185–189, 1992. doi: 10.1016/0165-3806(92)90178-y.

586. Stafstrom CE, Sasaki-Adams DM. NMDA-induced seizures in developing rats cause long-term learning impairment and increased seizure susceptibility. **Epilepsy Res** 53: 129–137, 2003.

doi: 10.1016/s0920-1211(02)00258-9.

587. Velísek L, Jehle K, Asche S, Velísková J. Model of infantile spasms induced by N-methyl-D-aspartic acid in prenatally impaired brain. **Ann Neurol** 61: 109–119, 2007. doi: 10.1002/ana.21082.

588. Kim E-H, Yum M-S, Lee M, Kim E-J, Shim W-H, Ko T-S. A New Rat Model of Epileptic Spasms Based on Methylazoxymethanol-Induced Malformations of Cortical Development. **Front Neurol** 8: 271, 2017. doi: 10.3389/fneur.2017.00271.

589. Rensing N, Johnson KJ, Foutz TJ, Friedman JL, Galindo R, Wong M. Early developmental electroencephalography abnormalities, neonatal seizures, and induced spasms in a mouse model of tuberous sclerosis complex. **Epilepsia** 61: 879–891, 2020. doi: 10.1111/epi.16495.

590. Cortez MA, Shen L, Wu Y, Aleem IS, Trepanier CH, Sadeghnia HR, et al. Infantile spasms and Down syndrome: a new animal model. **Pediatr Res** 65: 499–503, 2009. doi: 10.1203/PDR.0b013e31819d9076.

591. Joshi K, Shen L, Michaeli A, Salter M, Thibault-Messier G, Hashmi S, et al. Infantile spasms in down syndrome: Rescue by knockdown of the GIRK2 channel. **Ann Neurol** 80: 511–521, 2016. doi: 10.1002/ana.24749.

592. Frost JD, Le JT, Lee CL, Ballester-Rosado C, Hrachovy RA, Swann JW. Vigabatrin therapy implicates neocortical high frequency oscillations in an animal model of infantile spasms. **Neurobiol Dis** 82: 1–11, 2015. doi: 10.1016/j.nbd.2015.04.019.

593. Frost JD, Lee CL, Hrachovy RA, Swann JW. High frequency EEG activity associated with ictal events in an animal model of infantile spasms. **Epilepsia** 52: 53–62, 2011. doi: 10.1111/j.1528-1167.2010.02887.x.

594. Frost JD, Lee CL, Le JT, Hrachovy RA, Swann JW. Interictal high frequency oscillations in an animal model of infantile spasms. **Neurobiol Dis** 46: 377–388, 2012. doi:

10.1016/j.nbd.2012.02.001.

595. Price MG, Yoo JW, Burgess DL, Deng F, Hrachovy RA, Frost JD, et al. A triplet repeat expansion genetic mouse model of infantile spasms syndrome, Arx(GCG)10+7, with interneuronopathy, spasms in infancy, persistent seizures, and adult cognitive and behavioral impairment. **J Neurosci** 29: 8752–8763, 2009. doi: 10.1523/JNEUROSCI.0915-09.2009.

596. Pirone A, Alexander J, Lau LA, Hampton D, Zayachkivsky A, Yee A, et al. APC conditional knock-out mouse is a model of infantile spasms with elevated neuronal $β$-catenin levels, neonatal spasms, and chronic seizures. **Neurobiol Dis** 98: 149–157, 2017. doi: 10.1016/j.nbd.2016.11.002.

597. Gataullina S, Lemaire E, Wendling F, Kaminska A, Watrin F, Riquet A, et al. Epilepsy in young Tsc1(+/-) mice exhibits age-dependent expression that mimics that of human tuberous sclerosis complex. **Epilepsia** 57: 648–659, 2016. doi: 10.1111/epi.13325.

598. Mulcahey PJ, Tang S, Takano H, White A, Davila Portillo DR, Kane OM, et al. Aged heterozygous Cdkl5 mutant mice exhibit spontaneous epileptic spasms. **Exp Neurol** 332: 113388, 2020. doi: 10.1016/j.expneurol.2020.113388.

599. Chan KFY, Burnham WM, Jia Z, Cortez MA, Snead OC. GABAB receptor antagonism abolishes the learning impairments in rats with chronic atypical absence seizures. **Eur J Pharmacol** 541: 64–72, 2006. doi: 10.1016/j.ejphar.2006.04.012.

600. Chan KFY, Jia Z, Murphy PA, Burnham WM, Cortez MA, Snead OC. Learning and memory impairment in rats with chronic atypical absence seizures. **Exp Neurol** 190: 328–336, 2004. doi: 10.1016/j.expneurol.2004.08.001.

601. Han HA, Cortez MA, Snead OC. GABAB Receptor and Absence Epilepsy [Online]. http://www.ncbi.nlm.nih.gov/pubmed/22787624.

602. Li H, Kraus A, Wu J, Huguenard JR, Fisher RS. Selective changes in thalamic and cortical GABAA receptor subunits in a model of acquired absence epilepsy in the rat. **Neuropharmacology** 51: 121–128, 2006. doi: 10.1016/j.neuropharm.2006.03.003.

603. Smith KA, Bierkamper GG. Paradoxical role of GABA in a chronic model of petit mal (absence)-like epilepsy in the rat. **Eur J Pharmacol** 176: 45–55, 1990. doi: 10.1016/0014-2999(90)90130-x.

604. Smith KA, Fisher RS. The selective GABAB antagonist CGP-35348 blocks spike-wave bursts in the cholesterol synthesis rat absence epilepsy model. **Brain Res** 729: 147–150, 1996. http://www.ncbi.nlm.nih.gov/pubmed/8876982.

605. Stewart LS, Cortez MA, Snead OC. Environmental enrichment improves behavioral outcome in the AY-9944 model of childhood atypical absence epilepsy. **Int J Neurosci** 122: 449–457, 2012. doi: 10.3109/00207454.2012.677881.

606. Serbanescu I, Cortez MA, McKerlie C, Snead OC. Refractory atypical absence seizures in rat: a two hit model. **Epilepsy Res** 62: 53–63, 2004. doi: 10.1016/j.eplepsyres.2004.08.003.

607. Asinof S, Mahaffey C, Beyer B, Frankel WN, Boumil R. Dynamin 1 isoform roles in a mouse model of severe childhood epileptic encephalopathy. **Neurobiol Dis** 95: 1–11, 2016. doi: 10.1016/j.nbd.2016.06.014.

608. Wu Y, Chan KFY, Eubanks JH, Guin Ting Wong C, Cortez MA, Shen L, et al. Transgenic mice over-expressing GABA(B)R1a receptors acquire an atypical absence epilepsy-like phenotype. **Neurobiol Dis** 26: 439–451, 2007. doi: 10.1016/j.nbd.2007.01.013.

609. Stewart LS, Wu Y, Eubanks JH, Han H, Leschenko Y, Perez Velazquez JL, et al. Severity of atypical absence phenotype in GABAB transgenic mice is subunit specific. **Epilepsy Behav** 14: 577–581, 2009. doi: 10.1016/j.yebeh.2009.01.019.

610. Cox GA, Lutz CM, Yang CL, Biemesderfer D, Bronson RT, Fu A, et al. Sodium/hydrogen

exchanger gene defect in slow-wave epilepsy mutant mice. **Cell** 91: 139–148, 1997. doi: 10.1016/s0092-8674(01)80016-7.

611. Escayg A, Goldin AL. Sodium channel SCN1A and epilepsy: mutations and mechanisms. **Epilepsia** 51: 1650–1658, 2010. doi: 10.1111/j.1528-1167.2010.02640.x.

612. Cheah CS, Yu FH, Westenbroek RE, Kalume FK, Oakley JC, Potter GB, et al. Specific deletion of NaV1.1 sodium channels in inhibitory interneurons causes seizures and premature death in a mouse model of Dravet syndrome. **Proc Natl Acad Sci U S A** 109: 14646–14651, 2012. doi: 10.1073/pnas.1211591109.

613. Schutte RJ, Schutte SS, Algara J, Barragan E V, Gilligan J, Staber C, et al. Knock-in model of Dravet syndrome reveals a constitutive and conditional reduction in sodium current. **J Neurophysiol** 112: 903–912, 2014. doi: 10.1152/jn.00135.2014.

614. Baraban SC, Dinday MT, Hortopan GA. Drug screening in Scn1a zebrafish mutant identifies clemizole as a potential Dravet syndrome treatment. **Nat Commun** 4: 2410, 2013. doi: 10.1038/ncomms3410.

615. Pernici CD, Mensah JA, Dahle EJ, Johnson KJ, Handy L, Buxton L, et al. Development of an antiseizure drug screening platform for Dravet syndrome at the NINDS contract site for the Epilepsy Therapy Screening Program. **Epilepsia** 62: 1665–1676, 2021. doi: 10.1111/epi.16925.

616. Ihara Y, Tomonoh Y, Deshimaru M, Zhang B, Uchida T, Ishii A, et al. Retigabine, a Kv7.2/Kv7.3-Channel Opener, Attenuates Drug-Induced Seizures in Knock-In Mice Harboring Kcnq2 Mutations. **PLoS One** 11: e0150095, 2016. doi: 10.1371/journal.pone.0150095.

617. Vanhoof-Villalba SL, Gautier NM, Mishra V, Glasscock E. Pharmacogenetics of KCNQ channel activation in 2 potassium channelopathy mouse models of epilepsy. **Epilepsia** 59: 358–368, 2018. doi: 10.1111/epi.13978.

618. Rakotomamonjy J, Sabetfakhri NP, McDermott SL, Guemez-Gamboa A. Characterization of seizure susceptibility in Pcdh19 mice. **Epilepsia** 61: 2313–2320, 2020. doi: 10.1111/epi.16675.

619. Hoshina N, Johnson-Venkatesh EM, Hoshina M, Umemori H. Female-specific synaptic dysfunction and cognitive impairment in a mouse model of PCDH19 disorder. **Science** 372, 2021. doi: 10.1126/science.aaz3893.

620. Trovò L, Fuchs C, De Rosa R, Barbiero I, Tramarin M, Ciani E, et al. The green tea polyphenol epigallocatechin-3-gallate (EGCG) restores CDKL5-dependent synaptic defects in vitro and in vivo. **Neurobiol Dis** 138: 104791, 2020. doi: 10.1016/j.nbd.2020.104791.

621. Wang H-T, Zhu Z-A, Li Y-Y, Lou S-S, Yang G, Feng X, et al. CDKL5 deficiency in forebrain glutamatergic neurons results in recurrent spontaneous seizures. **Epilepsia** 62: 517–528, 2021. doi: 10.1111/epi.16805.

622. Insel TR, Miller LP, Gelhard RE. The ontogeny of excitatory amino acid receptors in rat forebrain--I. N-methyl-D-aspartate and quisqualate receptors. **Neuroscience** 35: 31–43, 1990. doi: 10.1016/0306-4522(90)90117-m.

623. Wang DD, Kriegstein AR. GABA regulates excitatory synapse formation in the neocortex via NMDA receptor activation. **J Neurosci** 28: 5547–5558, 2008. doi: 10.1523/JNEUROSCI.5599-07.2008.

3770 **Tables**

3771 **Table 1. Developmental and epileptic encephalopathies (DEEs) by age**

	Age of onset	Gender	Etiology	Electroclinical characteristics
Neonatal – Infantile onset				
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.
Dravet syndrome	3-9mo (1-20mo)	M=F	Genetic	***Clinical***: 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.

	Age of onset	Gender	Etiology	Electroclinical characteristics
				EEG: Focal or multifocal epileptiform abnormalities and seizures, photoparoxysmal responses.
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**	First year of life	M=F, M<F (*PCDH19*, *CDKL5*)	Genetic	***KCNQ2-DEE***: Sequential or focal tonic seizures, burst suppression; autonomic symptoms, epileptic spasms. Burst suppression or multifocal EEG. ***CDKL5-DEE***: Tonic seizures, epileptic spasms. hypermotor-tonic-spasms; movement disorders ***PDE, P5PDE***: Intrauterine or early life seizures; focal seizures, spasms or generalized tonic-clonic; response to pyridoxine or P5P. EEG: burst suppression or multifocal discharges. ***Glut1D***S: Intellectual disability, low CSF/plasma glucose ratio, generalized seizures (myoclonic, myoclonic-atonic, generalized tonic-clonic, absences). EEG: 2.5-5Hz spike-wave. **PCDH19-DEE**: Intellectual disability, autism spectrum disorder; focal impaired aware to tonic or atypical absence seizures. EEG: focal onset seizures. ***Sturge-Weber***: 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.

	Age of onset	Gender	Etiology	Electroclinical characteristics
- 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				
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).

	Age of onset	Gender	Etiology	Electroclinical characteristics
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.
<u>Variable age onset</u>				
Progressive myoclonus epilepsy (PME)				
- **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).
- **Lafora disease (EPM2)**	6-19y	M=F	Genetic	***Clinical:*** Vision loss, cognitive decline, cerebellar symptoms. Myoclonic and generalized tonic-clonic seizures. ***EEG:*** Photosensitivity; spike wave and polyspikes, no activation in sleep.

	Age of onset	Gender	Etiology	Electroclinical characteristics
- **Neuronal Ceroid Lipofuscinosis (NCL)**				
- **CLN2 (late infantile)**	2-4yo	M=F	Genetic	***Clinical:*** Language delay, progressive course, multiple seizures febrile and afebrile, including myoclonic. ***EEG:*** Photoparoxysmal response at low frequencies.
- **Juvenile CLN3**	4-10y	M=F	Genetic	***Clinical:*** Visual loss progressive, macular degeneration, optic atrophy, retinitis pigmentosa. ***EEG:***
- **Adult NCL (Kufs)**	11-50y	M=F	Genetic	***Type A:*** PME with dementia and ataxia. **Type B:** Dementia with cerebellar or extrapyramidal motor symptoms but not PME.

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.

3777 **TABLE 2. Genes involved in epileptic encephalopathy and DEE pathogenesis**

	Approved Symbol	Gene/Locus name
Ion channels		
	KCNA2	Potassium channel, voltage-gated, Shaker-related subfamily, member 2
	CACNA1E	Calcium channel, voltage-dependent, alpha 1E subunit
	KCNT2	Potassium channel, subfamily T, member 2
	SCN3A	Sodium channel, voltage-gated, type III, alpha polypeptide
	SCN2A	Sodium channel, voltage-gated, type II, alpha subunit
	SCN1A	Sodium channel, voltage-gated, type I, alpha polypeptide
	SCN9A	Sodium channel, voltage-gated, type IX, alpha subunit
	CACNA2D2	Calcium channel, voltage-dependent, alpha-2/delta subunit 2
	HCN1	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1
	KCNQ5	Potassium channel, voltage-gated, KQT-like subfamily, member 5
	KCNT1	Potassium channel, subfamily T, member 1
	CACNA1B	Calcium channel, voltage-dependent, L type, alpha 1B subunit
	SCN8A	Sodium channel, voltage gated, type VIII, alpha polypeptide
	CACNA1G	Calcium channel, voltage-dependent, T type, alpha-1G subunit
	CACNA1A	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
	SCN1B	Sodium channel, voltage-gated, type I, beta polypeptide
	KCNB1	Potassium voltage-gated channel, Shab-related subfamily, member 1
	KCNQ2	Potassium voltage-gated channel, KQT-like subfamily, member 2
	CLCN4	Chloride channel-4

	Approved Symbol	Gene/Locus name
Receptors		
	GABRA2	Gamma-aminobutyric acid (GABA) A receptor, alpha-2
	GABRB1	Gamma-aminobutyric acid (GABA) A receptor, beta-1
	GABRB2	Gamma-aminobutyric acid (GABA) A receptor, beta-2
	GABRA1	Gamma-aminobutyric acid (GABA) A receptor, alpha-1
	GABRG2	Gamma-aminobutyric acid (GABA) A receptor, gamma-2
	NTRK2	Neurotrophic tyrosine kinase, receptor, type 2
	GABBR2	Gamma-aminobutyric acid B receptor 2
	GRIN1	Glutamate receptor, ionotropic, N-methyl D-aspartate 1
	GABRB3	Gamma-aminobutyric acid (GABA) A receptor, beta-3
	GABRA5	Gamma-aminobutyric acid (GABA) A receptor, alpha-5
	GRIN2A	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A
	GRIN2D	Glutamate receptor, ionotropic, N-methyl-D-aspartate 2D
Transporters		
	ATP1A2	ATPase, Na+/K+ transporting, alpha-2 polypeptide
	SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1
	ARV1	ARV1 homolog, fatty acid homeostasis modulator
	SLC1A4	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
	SLC25A12	Solute carrier family 25 (mitochondrial carrier, Aralar), member 12
	SLC6A1	Solute carrier family 6 (neurotransmitter transporter, GABA), member 1
	SLC25A22	Solute carrier family 25 (mitochondrial carrier, glutamate), member 22
	SLC1A2	Solute carrier family 1 (glial high affinity glutamate transporter),

	Approved Symbol	Gene/Locus name
		member 2
	SLC13A5	Solute carrier family 13 (sodium-dependent citrate transporter), member 5
	SLC25A10	Solute carrier family 25 (mitochondrial carrier), member 10 (dicarboxylate ion carrier)
	SLC25A42	Solute carrier family 25, member 42
	ATP1A3	ATPase, Na+/K+ transporting, alpha-3 polypeptide
	SLC12A5	Solute carrier family 12, (potassium-chloride transporter) member 5
	SLC35A2	Solute carrier family 35 (UDP-galactose transporter), member 2
	SLC9A6	Solute carrier family 9 (sodium/hydrogen exchanger), member 6
Synapse related		
	CPLX1	Complexin 1
	PPP3CA	Protein phosphatase 3, catalytic subunit, alpha isoform (calcineurin A alpha)
	SYNGAP1	Synaptic Ras GTPase activating protein 1
	ADAM22	ADAM metallopeptidase domain 22
	STXBP1	Syntaxin-binding protein 1
	DNM1	Dynamin-1
	NECAP1	NECAP endocytosis-associated protein 1
	DMXL2	DMX-like 2
	AP3B2	Adaptor-related protein complex 3, beta 2 subunit
	STX1B	Syntaxin 1B
	SYNJ1	Synaptojanin 1
	NRXN1	Neurexin 1
Cell growth, division, and proliferation related		

	Approved Symbol	Gene/Locus name
	MTOR	Mechanistic target of rapamycin
	AKT3	AKT serine/threonine kinase 3
	NPRL2	NPR2-like protein, GATOR1 complex subunit
	STAG1	Stromal antigen 1
	RNF13	RING finger protein 13
	PIK3CA	Phosphatidylinositol 3-kinase, catalytic, alpha
	PPP2CA	Protein phosphatase-2 (formerly 2A), catalytic subunit, alpha isoform
	ACTL6B	Actin-like 6B
	RHOBTB2	Rho-related BTB domain-containing protein 2
	TSC1	Hamartin
	AKT1	AKT serine/threonine kinase 1
	NPRL3	Nitrogen permease regulator-like 3
	TSC2	Tuberin
	PIK3R2	Phosphatidylinositol 3-kinase, regulatory subunit 2
	DEPDC5	DEP domain-containing protein 5
Cell metabolism related		
	MTHFR	Methylenetetrahydrofolate reductase
	ST3GAL3	ST3 beta-galactoside alpha-2,3-sialyltransferase 3
	PARS2	Prolyl-tRNA synthetase 2
	HNRNPU	Heterogeneous nuclear ribonucleoprotein U
	CAD	CAD trifunctional protein of pyrimidine biosynthesis
	MDH1	Malate dehydrogenase, soluble
	UGP2	Uridyl diphosphate glucose pyrophosphorylase-2
	BOLA3	bolA family member 3
	ST3GAL5	Sialyltransferase 9

	Approved Symbol	Gene/Locus name
	GAD1	Glutamate decarboxylase-1, brain, 67kD
	GLS	Glutaminase
	D2HGDH	D-2-hydroxyglutarate dehydrogenase
	UGDH	UDP-glucose dehydrogenase
	MANBA	Mannosidase, beta A, lysosomal
	NUS1	NUS1 dehydrodolichyl diphosphate synthase subunit
	MDH2	Malate dehydrogenase, mitochondrial
	DENND5A	DENN domain-containing protein 5A
	NARS2	Asparaginyl-tRNA synthetase 2
	ALG9	ALG9 alpha-1,2-mannosyltransferase
	FCSK	Fucose kinase
	PNPO	Pyridoxamine 5'-phosphate oxidase
	ITPA	Inosine triphosphatase-A
	ALG13	ALG13 UDP-N-acetylglucosaminyltransferase subunit
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		
	SZT2	SZT2 subunit of KICSTOR complex
	DOCK7	Dedicator of cytokinesis 7
	YWHAG	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase

	Approved Symbol	Gene/Locus name
		activation protein, gamma isoform
	GNAO1	Guanine nucleotide-binding protein (G protein), alpha-activating activity
	PLCB1	Phospholipase C, beta-1
	SIK1	Salt-inducible kinase 1
Transcription and gene expression related		
	PUM1	Pumilio RNA binding family member 1
	TSEN2	tRNA splicing endonuclease, subunit 2
	PURA	Purine-rich element binding protein A
	MEF2C	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)
	KMT2E	Lysine (K)-specific methyltransferase 2E
	CELF2	CUGbp- and ELAV-like family, member 2
	CUX2	Cut-like homeobox 2
	FOXG1	Forkhead box G1B
	IRF2BPL	Interferon regulatory factor 2-binding protein like
	CHD2	Chromodomain helicase DNA binding protein-2
	NEUROD2	Neurogenic differentiation 2
	MECP2	Methyl-CpG-binding protein-2
Protein biosynthesis/degradation related		
	DHDDS	Dehydrodolichyl diphosphate synthase
	ATP6V1A	ATPase, H+ transporting, V1 subunit A
	UBA5	Ubiquitin-like modifier activating enzyme 5
	GUF1	GUF1 homolog, GTPase
	VARS1	Valyl-tRNA synthetase 1
	PLAA	Phospholipase A2-activating protein
	CARS2	Cysteinyl-tRNA synthetase 2

	Approved Symbol	Gene/Locus name
	AARS1	Alanyl-tRNA synthetase 1
	EEF1A2	Eukaryotic translation elongation factor-1, alpha-2
	PIGP	Phosphatidylinositol glycan, class P
	PIGA	Phosphatidylinositol glycan, class A
Cytoskeletal proteins		
	CYFIP2	Cytoplasmic FMRP interacting protein 2
	PHACTR1	Phosphatase and actin regulator 1
	SPTAN1	Spectrin, alpha, nonerythrocytic-1 (alpha-fodrin)
Mitochondria proteins		
	MFF	Mitochondrial fission factor
	FARS2	Phenylalanyl-tRNA synthetase 2, mitochondrial
	RMND1	Required for meiotic nuclear division 1 homolog
	BRAT1	BRCA1-associated ATM activator 1
	PMPCB	Peptidase, mitochondrial processing, beta
	TWNK	Twinkle mtDNA helicase
	DNM1L	Dynamin 1-like
	POLG	Polymerase (DNA directed), gamma
	GOT2	Glutamic-oxaloacetic transaminase 2, mitochondrial
	TIMM50	Translocase of inner mitochondrial membrane 50
Other/multiple functions proteins		
	FBXO11	F-box only protein 11
	MBD5	Methyl-CpG-binding domain protein 5
	PTPN23	Protein-tyrosine phosphatase, nonreceptor-type, 23
	DALRD3	DALR anticodon-binding domain-containing protein 3
	SERPINI1	Protease inhibitor 12
	FGF12	Fibroblast growth factor-12
	CSNK2B	Casein kinase-2, beta polypeptide

	Approved Symbol	Gene/Locus name
	CNPY3	Canopy 3, zebrafish, homolog of
	CDK19	Cyclin-dependent kinase 19
	TRRAP	Transformation/transcription domain-associated protein
	FRRS1L	Ferric-chelate reductase 1-like
	COQ4	Coenzyme Q4, S. cerevisiae, homolog of
	ATN1	Atrophin 1
	PACS2	Phosphofurin acidic cluster sorting protein 2
	PIGB	Phosphatidylinositol glycan, class B
	PIGQ	Phosphatidylinositol glycan anchor biosynthesis class Q protein
	ROGDI	Rogdi atypical leucine zipper
	WWOX	WW domain-containing oxidoreductase
	PIGS	Phosphatidylinositol glycan, class S
	TCF4	Transcription factor-4 (immunoglobulin transcription factor-2)
	PNKP	Polynucleotide kinase 3' phosphatase
	CDKL5	Cyclin-dependent kinase-like 5 (serine/threonine protein kinase 9)
	CASK	Calcium/calmodulin-dependent serine protein kinase
	SMC1A	Segregation of mitotic chromosomes 1 (SMC1, yeast, homolog of; DXS423E; SB1.8)
	FGF13	Fibroblast growth factor-13
	ARHGEF9	Rho guanine nucleotide exchange factor 9
	PCDH19	Protocadherin 19

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.

3783 **TABLE 3. Selected animal models of DEEs**

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
A. Models of epileptic spasms (ES)								
A1. Acute								
NMDA	Rat, mouse C57	NMDA i.p., PN7-18	PN7-18	NR	*Rat:* Learning and coordination deficits; *3days – 7days post NMDA (mouse):* Increased anxiety, impaired motor coordination and poor memory retention	High dose $ACTH_{1-39}$; Vigabatrin	Spasms	(167, 170, 543, 584–587)
NMDA variants								
- Prenatal betamethaso	Rat	Betamethasone i.p. G15;	PN12-15	NR	NR after spasms induction	Low dose $ACTH_{1-39}$;	Spasms, ACTH	(168, 537, 538, 542)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
ne / postnatal NMDA		NMDA i.p., PN12-15				Vigabatrin	sensitivity	
- Prenatal stress / postnatal NMDA	Rat	Forced restraint (FR) (G15 or Forced swim test (FST) (G1-parturition); NMDA i.p., PN15	PN15	NR	NR after spasms induction	FST/NMDA: Responds to $ACTH_{1-39}$; *FR/NMDA*: Responds to repeated low dose $ACTH_{1-39}$	Spasms, stress	(167, 169)
- Prenatal MAM / postnatal NMDA	Rat	MAM (2 doses, G15); NMDA i.p., PN12-15 (1 or 3 doses)	PN12-15	NR	NR after spasms induction	No effect of low dose ACTH; Responds to vigabatrin	Spasms, dysplasias	(588)
- Adrenalecto	Rat	Adrenalectomy (PN10); NMDA i.p.	PN11	NR	NR after spasms induction	High dose $ACTH_{1-39}$	Spasms	(170)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
my / postnatal NMDA		(PN11)						
- *Tsc1*$^{gfap\ -/+}$ mouse, postnatal NMDA,	Mouse, Tsc1$_{flox/flox}$-GFAP-Cre knockout C57Bl/6 and SV129			As in *Tsc1*$^{gfap\ -/+}$	NR after spasms induction	NR	Spasms, induced on a genetic background	(589)
Down syndrome / GBL	Mouse (Ts65Dn), C57Bl/ 6JEiXC3H/HesnJ	γ-butyrolactone (GBL) i.p.	1wk – 2mo	NR	NR after spasms induction	Responds to $ACTH_{1-24}$ but not to $ACTH_{1-39}$; Responds to vigabatrin	Spasms, induced on a genetic background	(539, 590, 591)
A2. Chronic								
Tetrodotoxin (TTX)	Rat	Intrahippocampal or intracortical TTX, unilateral	≥PN21	Yes	NR	Sensitive to ACTH, vigabatrin	ISS structural, hypsarrhyth	(87, 546, 592–594)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
		(PN10-38)					mia; drug-sensitive	
Multiple-hit	Rat	PN3: Right intracortical LPS, right intraventricular doxorubicin PN5: PCPA i.p.	PN4-13	Yes	Sociability deficits, learning/memory deficits, stereotypies	Resistant to ACTH; partial/transient response to vigabatrin	ISS structural, drug-resistant	(88, 155–161)
***Arx* cKO**	Mouse, CD1 and C57Bl/6	*Arx* deletion from ganglionic eminence neuronal progenitors	Adulthood	Yes	NR	NR	ISS, genetic	(162)
***Arx* KI [Arx $^{(GCG)10+7}$]**	Mouse, 75% C57BL/6; 25%	Expansion of 1st polyalalanine	PN7-11	Yes	Low anxiety, impaired learning	NR	ISS, genetic	(150, 163, 595)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
	129S5/SvEvBrd	tract repeat (PA1) of *Arx*			and sociability			
***Arx* with PA1 or PA2 expansion**	Mouse, C57BL/6N-Hsd	PA1 or PA2 expansion of *Arx*	≥PN10 to adulthood (myoclonic seizures)	NR	1-2mo: Sociability, neuromuscular strength deficits, anxiety and fear	NR	ISS, genetic	(545)
Adenomatous polyposis (*Apc*)	Mouse	*Apc* gene knockout from excitatory CamKII neurons	Peak at PN9	Yes	Reduced social interest, increased repetitive behaviors	NR	ISS, genetic	(596)
***Tsc1*+/-**	Mouse, C57BL/6	Heterozygous $Tsc1^{-/+}$	PN12-16; Observed for 1 day/pup	NR	NR	NR	ISS, genetic	(597)
Aged *CDKL5*, heterozygous females	Mouse, C57BL/6J	$Cdkl5^{R59X/+}$ or $Cdkl5^{KO/+}$	>PN300 female	No (only spasms seen)	NR in females; Sociability deficits in males	NR	ISS, genetic	(598)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
Chronic early life stress	Rat	Unpredictable and fragmented nurturing behaviors in dams (PN2-9 period)	PN17-35; Last for 1 or several days	NR	NR	NR	ISS, unknown	(171)
B. Models of Lennox-Gastaut syndrome, atypical absence seizures								
AY9944	Rat	AY9944 7.5mg/kg SC (PN2, 8, 14, PN20)	NR	Slow SWD	Cognitive deficits, hyperactivity, anxiety, spatial learning, olfactory recognition deficits	Responsive to DZP, ETH, CGP35348; Worse with CZP, baclofen, γ-OH-butyrate	Chronic atypical absence seizures	(599–605)
MAM - AY9944	Rat	Prenatal MAM / postnatal AY9944	NR	Slow SWD	NR	Refractory to ETH, VPA, CGP35348, CBZ	Refractory atypical absence seizures	(606)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
PValb-Dnm1$^{Ftfl/flox}$	Mouse, C57BL/6J	*Dnm1$^{Ftfl/flox}$* in PV cells	NR	PN19-50: SWD, lethal seizures	Tremor	NR	LGS	(607)
GABABR1a	Mouse, C57BL/6	*GABABR1a* overexpression in forebrain	NR	Slow SWD	Impairment in learning, spatial memory	Responsive to ETH, CGP35348	Chronic atypical absence seizures	(608)
GABABR1b	Mouse	*GABABR1b* overexpression in forebrain	NR	Slow SWD	Mild impairment in learning, spatial memory	Responsive to ETH, CGP35348	Chronic atypical absence seizures	(609)
NHE1	Mouse, SJL/J and C57BL/6J	Na+/H+ exchanger null	NR	Slow SWD (3Hz) (at 4-5wk); Lethal tonic or tonic-clonic	Ataxia; Early mortality	NR	Chronic atypical absence seizures	(610)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
				seizures				
Multiple-hit	Rat	PN3: Right intracortical LPS, right intraventricular doxorubicin PN5: PCPA i.p.	PN4-13	Adulthood: slow SWD (5-6Hz), motor seizures in sleep	Sociability deficits, learning/memory deficits, stereotypies	NR	ISS with LGS features	(155)
C. Models of Dravet syndrome								
***Scn1a* KO**	Mouse	Exon 26 deletion, global constitutive		Convulsive seizures, Hyperthermia seizures, mortality	Hyperactivity, stereotypies, sociability, and spatial memory deficits	Tested	Dravet	(64)
***Scn1a* CKO**	Mouse, C57BL/6 and	Exon 25 deletion,		Motor	NR		Dravet	(611)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
	129Sv	forebrain GABAergic interneurons		seizures, Hyperthermia seizures				
***Scn1a* CKO**	Mouse, B6.SJL-Tg(ACTFLPe)9205Dym/J and C57BL/6	Conditional deletion of exon 7	Inhibitory neurons	≥PN16 seizures, occasional death	Hypoactive, jerks, death		Dravet	(612)
			Forebrain excitatory neurons	No	NR			(612)
			Forebrain excitatory neurons and haploinsufficiency in inhibitory	Improved lethality from seizures	NR		Dravet	(612)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
			neurons					
			PV interneurons	≥PN14 seizures, death	Ataxia (PN10)		Dravet	(612)
***Scn1a* KI, R1407X**	Mouse, 129/SvJ and C57BL/6J	Human R1407X nonsense mutation		≥1mo: Seizures	Hyperactivity, stereotypies, sociability and		Dravet	(65, 224, 225)
					spatial memory deficits			
***Scn1a* KI, S1231R**	Drosophila	S1231R mutation, loss of function		Seizures	NR		Dravet	(613)
Scn1Lab (didy^{s552})	Zebrafish	Scn1Lab mutation (low expression)		Increased locomotor activity, epileptiform activity,	Impaired exploration, decreased mobility	Tested	Dravet	(614)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
				seizures				
Scn1Lab $^{-/-}$	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 R1648H missense mutation, global constitutive,*		Convulsive seizures, Hyperthermia seizures, mortality	Hyperactivity, stereotypies, sociability, and spatial memory deficits		Dravet and GEFS+	(228)
D. Other etiology-								

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
specific models of DEE								
Kcnq2 KI	Mouse, C57BL/6J	Kcnq2-Y284C/+, Kcnq2-A306T/+		NR	NR	Retigabine reduces KA-seizures	KCNQ2 DEE	(616)
***Kcnq2*-Thr274Met/+**	Mouse, 129Sv, C57BL/6N	Kcnq2-Thr274Met/+		Yes (>PN20)	Spatial learning and memory deficits. Death by 3rd mo (25%)	NR	KCNQ2 DEE	(281)
Kcna1-/-	Mouse	*Kcna1-/-*		Yes	NR	Retigabine reduces	KCNA1 epilepsy	(617)
						spontaneous seizures		
***Kcnq1*-**	Mouse	*Kcnq1*-		Rare	NR	Retigabine:	KCNQ1	(617)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
A340E/A340E		*A340E/A340E*		spontaneous seizures		adverse cardiac effects	epilepsy	
Pcdh19 KO and heterozygous females	Mouse, 129S5.C57BL/6	*Pcdh19 KO and heterozygous females*		NR; Increased susceptibility to 6Hz and flurothyl seizures		NR	*PCDH19 DEE*	(618)
Pcdh19-HET	Mouse, C57BL/6N	*Pcdh19-HET*	Mossy fiber deficits	NR	Pattern completion and separation deficits	NR	*PCDH19 DEE*	(619)
Pcdh19 KO	Mouse, C57BL/6N	*Pcdh19-KO*		NR	Increased exploratory behavior, reduced anxiety	NR	*PCDH19 DEE*	(618)

Models	Species	Induction method	Spasms, age of onset	Subsequent epilepsy	Behavioral / neurodevelopmental deficits	Response to ACTH/vigabatrin	Model of:	References
Cdkl5 CKO	Mouse, CD1	*Cdkl5 CKO in glutamatergic or GABAergic neurons*	Defective dendritic arborisation and spine maturation	Yes when deleted in glutamatergic neurons.	Autistic symptomatology, motor coordination, memory and breathing abnormalities	Epigallatocathechin-3-gallate (EGCG) corrects synaptic deficits	*CDKL5 DEE*	(620, 621)

ACTH: adrenocorticotropic hormone; Apc: adenomatous polyposis colon; Arx : aristaless X-linked homeobox protein; AY9944; trans-1,4-bis-cyclohexane dihydrochloride, cholesterol biosynthesis inhibitor; CDKL5: cyclin-dependent kinase-like 5; CGP35348: GABAB receptor inhibitor; DEE: DEE: Developmental and epileptic encephalopathy; ES: epileptic spasms; CKO: conditional knockout; CZP: clonazepam; Dnm1: dynamin 1; DZP: diazepam; EGCG: Epigallatocathechin-3-gallate; ETH: ethosuximide; GBL: gamma butyrolactone; FR: forced restraint; FST: Forced swim test; G: gestational day; GABABR: GABAB receptor; Gfap: glial acidic fibrillary protein; HET: heterozygous; ISS: infantile spasms syndrome; Kcna1: Potassium Voltage-Gated Channel Subfamily A Member 1); KCNQ: Potassium Voltage-Gated Channel Subfamily Q; KI: knockin; KO: knockout; LGS: Lennox-Gastaut syndrome; LPS: lipopolysaccharide; MAM: methyl-azoxy-methanol acetate; NHE1: Na+/H+ exchanger; NMDA: N-methyl-D-

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.

3793 **TABLE 4. Experimental drugs tested in models of DEE**

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
A. ES models					
CGP35348	$GABA_BR$ antagonism	Mouse, Down/GBL	Pre-treatment	Shortens EDRs	(538)
Baclofen	$GABA_BR$ agonist	Mouse, Down/GBL	Pre-treatment	Prolongs EDRs	(538)
5-OH-tryptophan	Serotonin increase	Mouse, Down/GBL	Pre-treatment	Prolongs EDRs	(538)
GIRK2 knockout	Deletion of $GABA_BR$ associated inward rectifying potassium channel	GIRK2 KO Mouse, Down/GBL	GIRK2 Knockout mice	GIRK2 knockout confers resistance to GBL induced spasms	(539)
Rapamycin	mTOR inhibitor	Rat, Prenatal betamethasone / postnatal NMDA	Pre-treatment	No effect, no evidence of target relevance	(537)
Ganaxolone	Allosteric	Rat, Prenatal	Pre-treatment	Reduces number,	(540)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
	activator of $GABA_AR$, synaptic and extrasynaptic	betamethasone / postnatal NMDA		delays onset of NMDA spasms	
ACTON PROLONGATUM ®	Synthetic ACTH based on porcine ACTH	Rat, Prenatal betamethasone / postnatal NMDA	Pre-treatment	Reduces number, delays onset of NMDA spasms after 2doses but not after single dose	(541)
AQB-565	ACTH1-24 linked to melanocyte stimulating hormone, acts on MC3, MC4 melanocortin receptors	Rat, Prenatal betamethasone / postnatal NMDA	Pre-treatment	Reduces number of NMDA spasms after 8 doses	(538)
Estradiol, diethylstilbestrol	Gonadal hormone,	Rat, Prenatal betamethasone /	Pre-treatment (PN3-10)	No effect on spasms; increased	(542)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
	estrogen analogue	postnatal NMDA		GAD67 cells in neocortex	
β-OH-butyrate	Ketoacid	Rat, Prenatal betamethasone / postnatal NMDA	Pre-treatment	Reduces spasms and delays latency to NMDA spasms after repeat but not single dose administration	(543)
β-OH-butyrate	Ketoacid	Rat, Prenatal betamethasone / postnatal NMDA	Pre-treatment (200mg/kg ip)	No effect	(544)
2-deoxyglucose	Metabolic inhibition of glycolysis	Rat, Prenatal betamethasone / postnatal NMDA	Pre-treatment	No effect	(544)
B. ISS models					
Rapamycin, pulse	mTOR inhibitor	Rat, multiple hit	Treatment after spasms onset, 3days	Decreases spasms within 2h; stops	(155, 161)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
				spasms with repeat dosing; improves spatial learning; prevents adult epilepsy; reverses mTOR dysregulation	
CPP-115	High affinity vigabatrin analog	Rat, multiple hit	Treatment after spasms onset, repeated (PN4-12)	Reduces spasms from the first hour and for up to 3 days; better efficacy and tolerability than vigabatrin	(156)
Carisbamate	Unknown; effect on spasms not due to sodium channel blockade	Rat, multiple hit	Treatment after spasms onset, single dose (PN4 or PN6-7)	Reduces spasms within the first hour	(160)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
NAX-5055	Galanin receptor 1 (GalR1) agonist	Rat, multiple hit	Treatment after spasms onset, single dose (PN4 or PN6-7)	No effect; low expression of GalR1 in pups	(158)
VX-765	Caspase 1 inhibitor	Rat, multiple hit	Treatment after spasms onset, single dose (PN4)	No effect	(157)
CGP 35348	$GABA_BR$ antagonism	Rat, multiple hit	Treatment after spasms onset, single dose (PN4)	No effect	(157)
17β-estradiol	Gonadal hormone	Rat, multiple hit	Treatment started after induction (PN3-10)	No effect	(157)
17β-estradiol	Gonadal hormone	Mouse, *Arx* KI [Arx (GCG^{10+7})	Pre-treatment (PN3-10)	Prevents spasms and other	(150)
				seizures, restores interneuronopathy	
17β-estradiol	Gonadal hormone	Mouse, Arx with PA1 or PA2 expansion	Pre-treatment (PN3-10)	Reduces seizures but not the	(545)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
				abnormal behavior	
C. Dravet syndrome models					
Soticlestat	Cholesterol 24-hydroxylase inhibitor	Mouse, Scn1a^{tm1Kea} with exon 1 deletion	Treatment after hyperthermia priming	Reduced seizures, protected against hyperthermia seizures, prevented SUDEP	(549)
Medium chain triglyceride diet (decanoic C10, octanoic acid C8 mix)	Ketogenic diet metabolite	*Mouse, Scn1a* KI, R1407X	4wk treatment prior to hyperthermia	C10/C8 (80:20) reduce both seizures and mortality; C10 reduces mortality	(551)
***Gabra2* repair**	*GABRA2* expression restoration (increase)	*Mouse, Scn1a*$^{+/-}$	Genetic repair of *Gabra2*	Rescues epilepsy phenotype	(550)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
***Trpv1* receptor deletion**	*Trpv1 receptor deletion*	*Mouse, Scn1a*$^{+/-}$	*Trpv1 receptor deletion*	No effects on hyperthermia seizures, frequency of spontaneous seizures or survival	(552)
SB-705498	*Trpv1* selective antagonist	*Mouse, Scn1a*$^{+/-}$		No effect on seizures or survival	(552)
Cannabigerolic acid	*Phytocannabinoid*	*Mouse, Scn1a*$^{+/-}$	Pre-treatment	Potentiated clobazam effects on hyperthermia induced and spontaneous seizures,	(554)
				anticonvulsant in MES, proconvulsant in 6Hz test.	

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
Cannabichromene, 5-Fluoro-Cannabichromene	*Phytocannabinoid*	*Mouse, Scn1a*$^{+/-}$	Pre-treatment	Anticonvulsant	(553)
Ketogenic diet	Ketogenic diet	*Mouse, Scn1a* KI, R1407X	Ketogenic diet, 14 days	Decreases SUDEP, protects against seizure induced respiratory arrest	(555)
SCN1A transfer in the brain (adenoviral)	SCN1A expression in the brain	Mouse, SCN1A-A1783V KI	Adenovirus expressing SCN1A, intracerebral injection	Protected from death, attenuation of epilepsy, hyperactivity persisted, cognitive effects variable	(556)
Naltrexone	Opioid antagonist	Zebrafish, *scn1Lab*	Pretreatment, 30min prior to PTZ	Anticonvulsant effects	(559)
Fenfluramine, Norflenfluramine	Reuptake inhibitor of 5-OH-	Zebrafish, *scn1Lab-/-*	Treatment exposure	Anticonvulsant effects	(560)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
enantiomers	tryptamine				
Fenfluramine	Reuptake inhibitor of 5-OH-tryptamine	Zebrafish, *scn1Lab*	Treatment exposure	Anticonvulsant effects	(561)
Dimethadione	Calcium channel blocker	Zebrafish, *scn1Lab*	Treatment exposure	Anticonvulsant effects	(561)
Clemizole	Serotoninergic	Zebrafish, *scn1Lab*	Treatment exposure	Anticonvulsant effects	(562)
PK11195	Pck1 activator and translocator protein ligand	Zebrafish, *scn1Lab*	Treatment exposure	Anticonvulsant effects	(563)
GR-46611	5HT1D receptor agonist	Mouse, 129S-Scn1Atm1Kea/Mmjax	Pretreatment	Protects from hyperthermia seizures, decreases seizure severity, improves survival	(557)
ASO, increases	ASO, increases	Mouse, F1:129S-	PN2 or 14,	Reduces seizures	(233)

Drug	Mechanism	Model / species	Treatment Protocol	Effect	Reference
Scn1a	*Scn1a (TANGO method)*	Scn1a+/− Å~ C57BL/6J	intracerebroventricular injection	and SUDEP	
CRISPR/dCas9-based *Scn1a* gene activation in inhibitory neurons	CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons	Mouse, *Scn1a*$^{Rx/+}$, floxed dCas9-VPR$^{VPR/+}$, Vgat-Cre$^{Cre/+,}$	Intravenous AAV injection, 4wk	Improved behavioral deficits, ameliorated febrile seizures	(558)
MV1369, MV1312	NaV1.6 inhibitor	Zebrafish, *Scn1Lab* KO	Treatment	Reduced spontaneous burst movements and seizures	(564)
AA43279	Nav1.1 activator	Zebrafish, *Scn1Lab* KO	Treatment	Reduced spontaneous burst movements and seizures	(564)

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: ectrodecremental responses; GABRA2: $GABA_AR$ A2 subunit; CGP35348: $GABA_B$ receptor inhibitor; DEE: Developmental and epileptic encephalopathy; ES: epileptic spasms; $GABA_BR$: $GABA_B$ receptor; GalR:

galanin receptor; GBL: gamma butyrolactone; GIRK: inward rectifying potassium channel; 5-HT: serotonin; KI: 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.

3802 **TABLE 5 Main drugs used to treat DEEs and target pathways/molecules**

	Excitatory transmission	Na^+ channels	Ca^{++} channels	K^+ channels	Glutamate receptors	5-HT receptors	Sigma-1 receptors	Inhibitory transmission	GABA-A receptors	GABA turnover	GABA-B receptors	SV2A binding	Carbonic anhydrase	Clinical efficacy
Barbiturates								YES	YES					
Benzodiazepines								YES	YES					BS
Brivaracetam												YES		FS
Cannabidiol								YES						LGS, DS
Carbamazepine	YES	YES	YES											FS, GTCS
Cenobamate		YES						YES	YES					FS (adult)
Clobazam	YES							YES	YES					LGS, FS
Eslicarbazepine	YES	YES												FS
Ethosuximide	YES		YES											ABS
Felbamate	YES	YES	YES		YES			YES	YES					BS
Fenfluramine	YES					YES		YES						DS
Gabapentin	YES		YES					YES		YES	YES			FS, GTCS
Lacosamide	YES	YES											YES	FS, SGS
Lamotrigine	YES	YES	YES											FS, GCTs,

	Excitatory transmission	Na^+ channels	Ca^{++} channels	K^+ channels	Glutamate receptors	5-HT receptors	Sigma-1 receptors	Inhibitory transmission	GABA-A receptors	GABA turnover	GABA-B receptors	SV2A binding	Carbonic anhydrase	Clinical efficacy
														ABS
Levetiracetam	YES		YES	YES				YES	YES			YES		BS
Oxcarbazepine	YES	YES	YES	YES										FS, GTCS
Perampanel	YES				YES									FS, GTCS
Phenobarbital	YES		YES		YES			YES	YES					FS
Phenytoin	YES	YES	YES											FS, GTCS
Pregabalin	YES		YES					YES						FS
Primidone	YES		YES					YES	YES					FS, GTCS
Retigabine	YES			YES										FS
Rufinamide	YES	YES												LGS
Stiripentol								YES	YES					DS
Tiagabine								YES		YES				FS
Topiramate	YES	YES	YES	YES	YES			YES	YES				YES	BS
Valproate	YES	YES	YES					YES	YES	YES				BS
Vigabatrin								YES		YES				FS, ES

	Excitatory transmission	Na^+ channels	Ca^{++} channels	K^+ channels	Glutamate receptors	5-HT receptors	Sigma-1 receptors	Inhibitory transmission	GABA-A receptors	GABA turnover	GABA-B receptors	SV2A binding	Carbonic anhydrase	Clinical efficacy
Zonisamide	YES	YES	YES										YES	FS, GTCs, MYO

3803 BS: broad spectrum; GABA:Gamma-amonobutyric acid; 5-TH: 5-hydroxytryptamine; SV2A: synaptic vesicle protein 2°; FS: focal seizures; LGS: Lennox Gastaut syndrome; DS:

3804 Dravet syndrome; GTCS: generalized tonic-clonic seizures; ABS: absence; SGS: secondary generalized seizures; ES: epileptic spasms; MYO: myoclonic.

3805 Adapted from https://www.uptodate.com/contents/antiseizure-drugs-mechanism-of-action-pharmacology-and-adverse-effects.

3806

3807 **TABLE 6. Clinical trials on DEEs.**

1	**Study Title:** Study to Evaluate NBI-921352 as Adjunctive Therapy in Subjects With SCN8A Developmental and Epileptic Encephalopathy Syndrome (SCN8A-DEE) **Recruiting:** Not yet **Has results:** No **Conditions:** SCN8A DEE Syndrome **Interventions:** Drug: NBI-921352, Placebo **Locations:** Neurocrine Clinical Site, Washington, District of Columbia, United States
2	**Study Title:** XEN496 (Ezogabine) in Children With KCNQ2 Developmental and Epileptic Encephalopathy **Recruiting:** Yes **Has results:** No **Conditions:** Epilepsy, Epilepsy in Children, Epilepsy; Seizure Disease, Brain Diseases, Central Nervous System Diseases, Nervous System Diseases. Epileptic Syndromes **Interventions:** Drug: XEN496, Placebo **Locations:** Children's Hospital of Colorado, Aurora, Colorado, United States. Northwest Florida Clinical Research Group, Gulf Breeze, Florida, United States. Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, United States. Columbia University Irving Medical Center, New York, New York, United States. The Cleveland Clinic Foundation, Cleveland, Ohio, United States. Oregon Health and Science University, Portland, Oregon, United States. MultiCare Health System - Mary Bridge Pediatrics - Tacoma, Tacoma, Washington, United States. Sydney Children's Hospital, Sydney, New South Wales, Australia. Children's Health Queensland Hospital and Health Service, South Brisbane, Queensland, Australia. Austin Health, Heidelberg, Victoria, Australia.

3	**Study Title:** An Open-Label Extension of the Study XEN496 (Ezogabine) in Children With KCNQ2-DEE **Recruiting:** Yes **Has results:** No **Conditions:** Epilepsy, Epilepsy in Children, Seizure Disease, Brain Diseases, Central Nervous System Diseases, Nervous System Diseases, Epileptic Syndromes **Interventions:** Drug: XEN496, Placebo **Locations:** MultiCare Health System - Mary Bridge Pediatrics - Tacoma, Tacoma, Washington, United States.
4	**Study Title:** Study of TAK-935 as an Adjunctive Therapy in Participants With Developmental and/or Epileptic Encephalopathies **Recruiting:** No **Has results:** Yes **Conditions:** Developmental and/or Epileptic Encephalopathies **Interventions:** Drug: TAK-935, Placebo **Locations:** 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.
5	**Study Title:** 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 **Recruiting:** No

	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, Bear 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 Dzieciecej i Leczenia Padaczki, 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

	Maternidade de Julio DinisCentro Materno Infantil do Norte, Porto, Portugal. Clinica Universidad Navarra, Pamplona, Navarra, Spain. Hospital Vithas La Salud, Granada, Spain. Hospital Ruber Internacional, Madrid, Spain. Hospital Universitari i Politecnic La Fe de Valencia, Valencia, Spain.

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

3858 (91). Brain growth spurt in these studies included gross brain growth, DNA, cholesterol, and water
3859 content. Puberty onset occurs around PN32-36 in female rats and PN35-45 in male rats, whereas
3860 in humans it starts around 10-11 years in girls and 11-12 years in boys (92). Distinct processes,
3861 such as neurogenesis and migration, synaptogenesis and synaptic pruning, myelination follow
3862 different time courses (90, 93–100). However, the staging of the equivalence of developmental
3863 stages across species is only approximate and each developmental process needs to be considered
3864 individually. **B**. Significant changes occur during development in the expression or function or
3865 various signaling processes. A schematic depiction of the age-related changes in $GABA_AR$ and
3866 glutamatergic signaling in rats is presented here, however cell type, regional and sex-specific
3867 differences also exist (90, 105, 622). Early in development, there is less effective $GABA_AR$-mediated
3868 inhibition, due to the presence of depolarizing $GABA_AR$ signaling (see also **FIGURE 4**), more tonic
3869 and less phasic $GABA_AR$ inhibition. In contrast, glutamatergic receptors, such as NMDAR or kainate
3870 receptors also show age-related expression patterns.
3871
3872 **FIGURE 4. Depolarizing and hyperpolarizing $GABA_AR$ signaling in normal development and**
3873 **disease. A**. $GABA_AR$ signaling is depolarizing early in life due to the higher intracellular Cl-
3874 concentrations ($[Cl^-]i$) that force Cl- efflux upon $GABA_AR$ activation. Although the $GABA_AR$
3875 depolarizations render GABA inhibition less efficient, as it relies upon shunt inhibition, they are
3876 critical for normal brain development. $GABA_AR$ depolarizations may activate L-type voltage
3877 sensitive calcium channels and may release the Mg^{++} block of NMDARs, triggering intracellular Ca^{++}
3878 rises that are important for neuronal survival, migration, differentiation and integration (123, 130,
3879 132, 623). The $[Cl^-]i$ in immature neurons is a result of increased expression and/or activity of Cl^-
3880 importers, like NKCC1 (a $Na^+/K^+/Cl^-$ cotransporter) over Cl^- exporters, like KCC2 (a K^+/Cl^-
3881 cotransporter). The Na^+/K^+ 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 $GABA_AR$ 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 $GABA_AR$ signaling. Genetic variants, drugs, perinatal or postnatal insults that trigger precocious presence of hyperpolarizing $GABA_AR$ 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-compartments (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

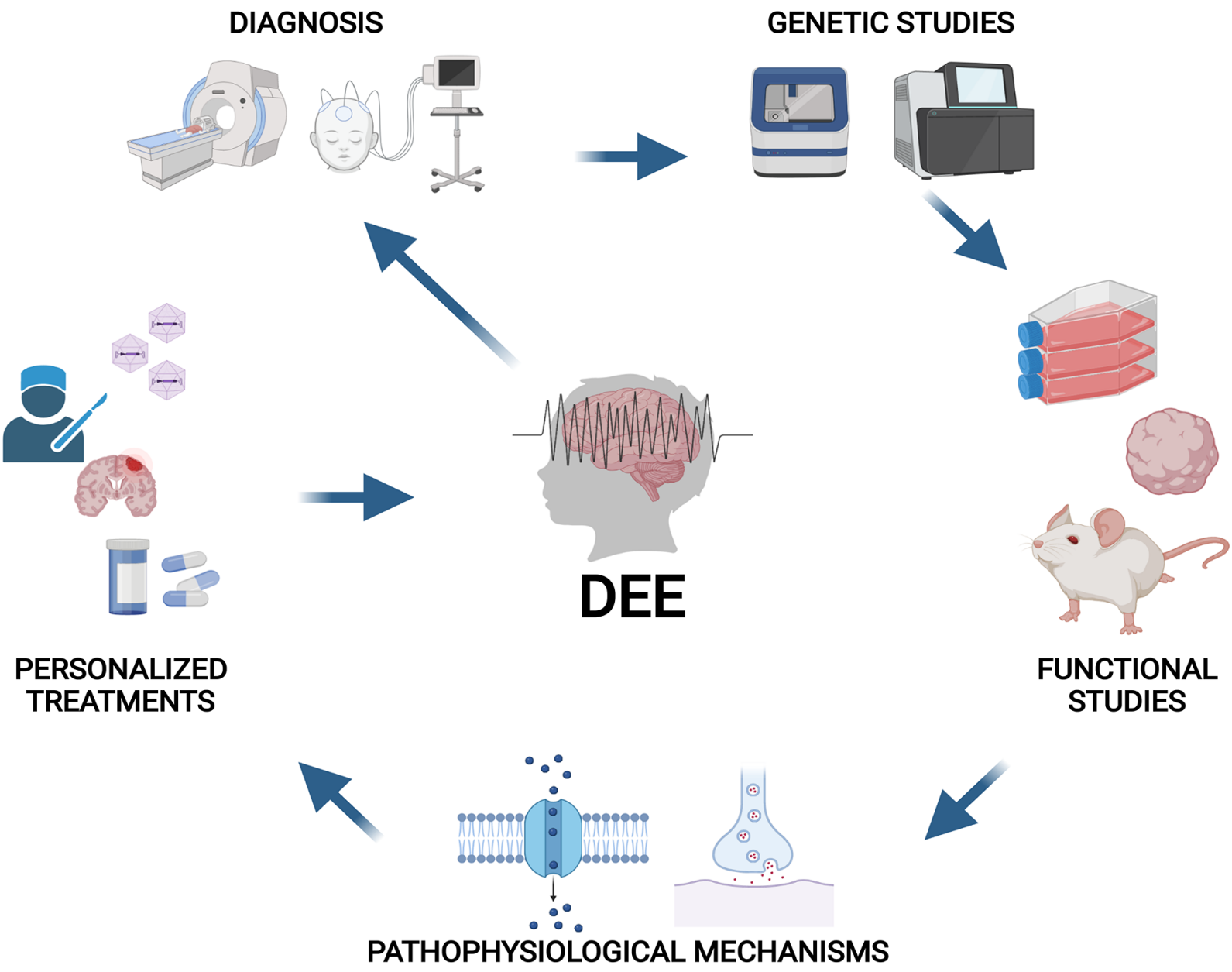
dysplasia with increased volume of the brain parenchyma, blurring of the gray-white matter junction and irregular cortical folding. **H**. T2W coronal section. Left parieto-temporal focal cortical dysplasia in a girl with *NPRL2* mutation. The circle surrounds the parietal portion of the cortical abnormality. **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

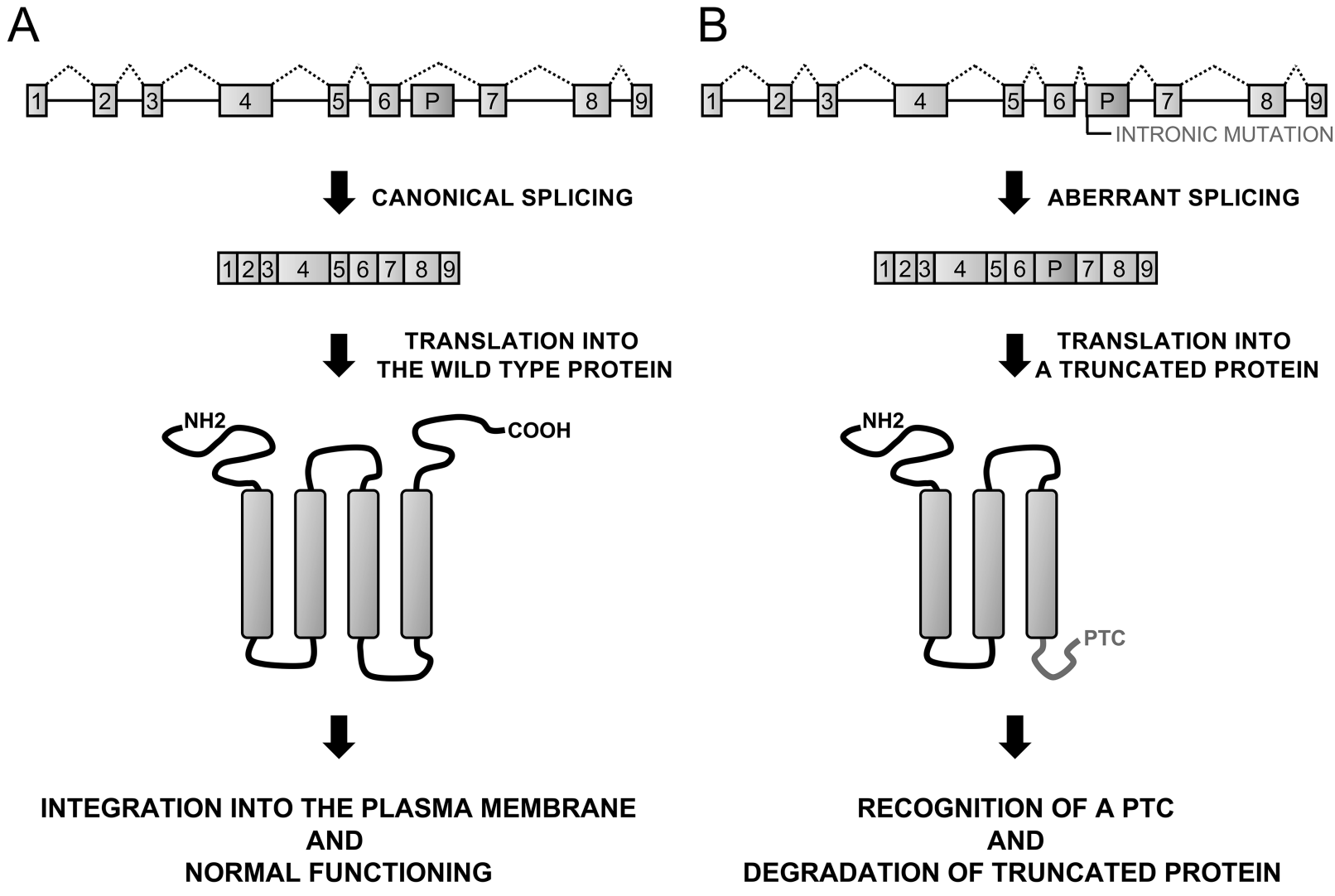
section. Polymicrogyria with abnormal cortical infoldings and packed microgyria (black arrows), combined with abnormal sulcation in a boy with *ATP1A3* mutation. **X.** T2W axial section. Bilateral frontoparietal cortical thickening and diffusely abnormal cortical pattern in a boy with biallelic *GPR56* 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

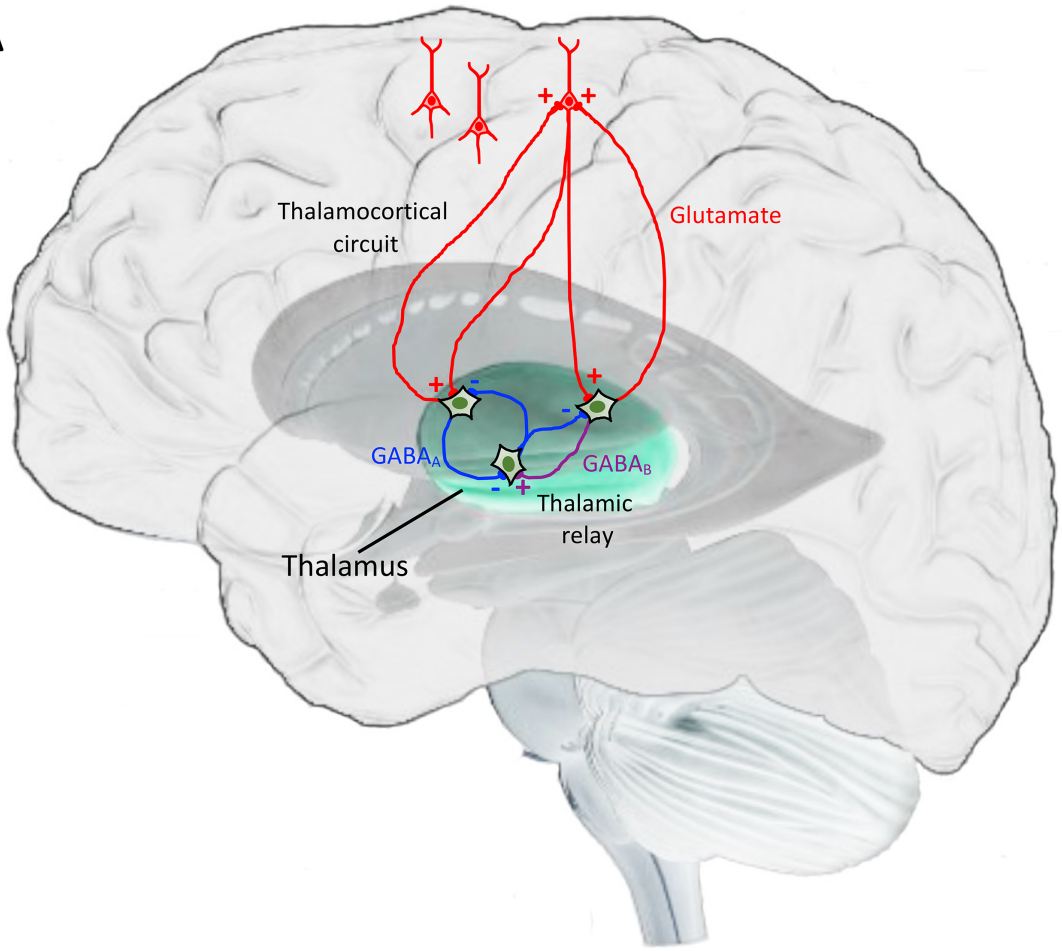
maintenance of SV pool in the nerve terminal and concentrates postsynaptic receptors on the postsynaptic side.

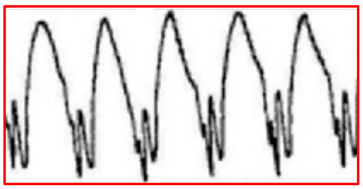
FIGURE 9. Schematic representation of the mTOR and autophagy intracellular cascades and their interrelationships. The complex regulatory cascade triggering the activation of the mTORC1 complex is initiated by extracellular signals (growth factors, neurotransmitter, hormones). To be activated, mTORC1 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.


DIAGNOSIS
GENETIC STUDIES
DEE
PERSONALIZED TREATMENTS
FUNCTIONAL STUDIES
PATHOPHYSIOLOGICAL MECHANISMS


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
NH2
COOH
INTEGRATION INTO THE PLASMA MEMBRANE
AND
NORMAL FUNCTIONING
B
1
2
3
4
5
6
P
7
8
9
INTRONIC MUTATION
ABERRANT SPLICING
1 2 3 4 5 6 P 7 8 9
TRANSLATION INTO
A TRUNCATED PROTEIN
NH2
PTC
RECOGNITION OF A PTC
AND
DEGRADATION OF TRUNCATED PROTEIN

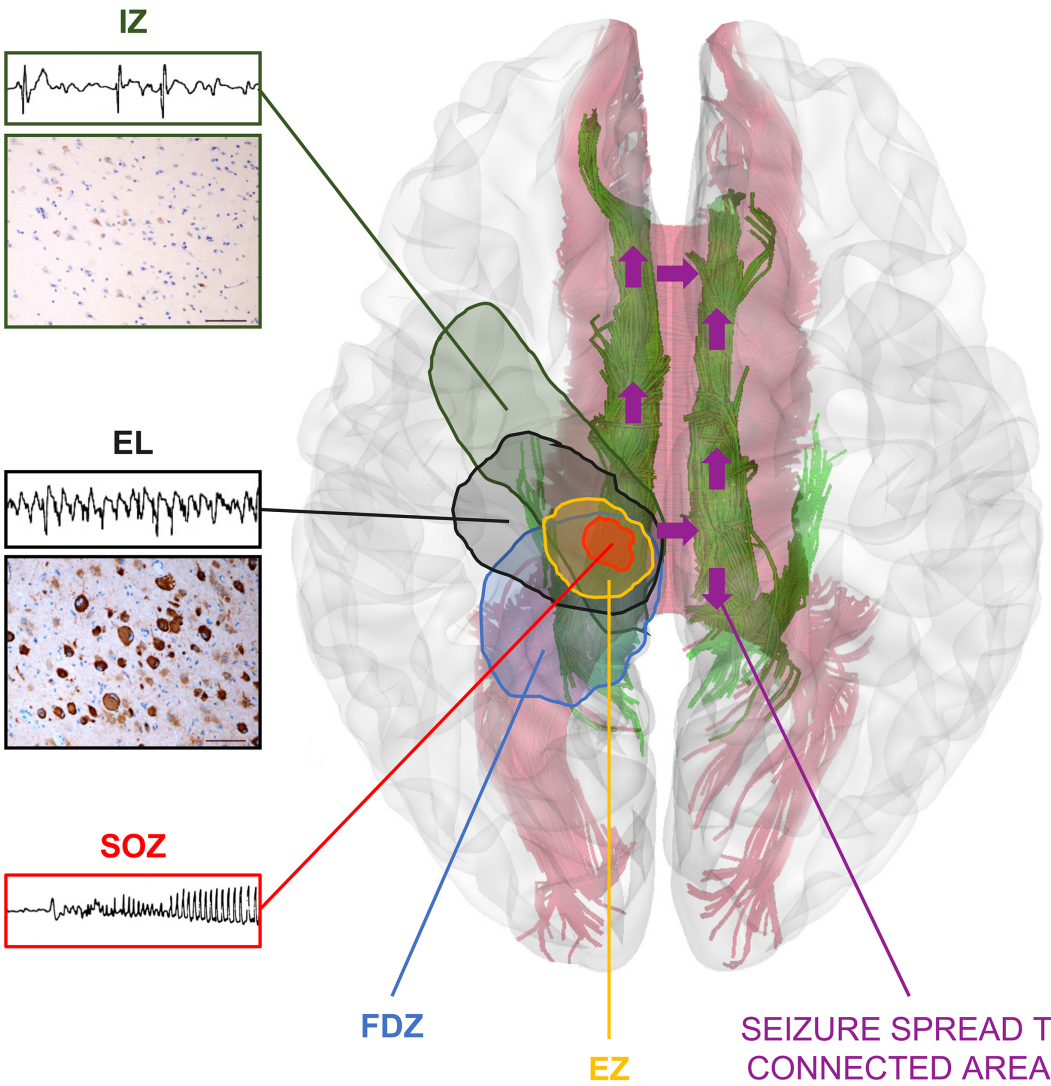
A

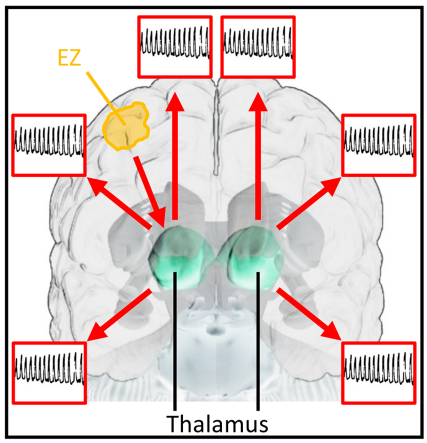




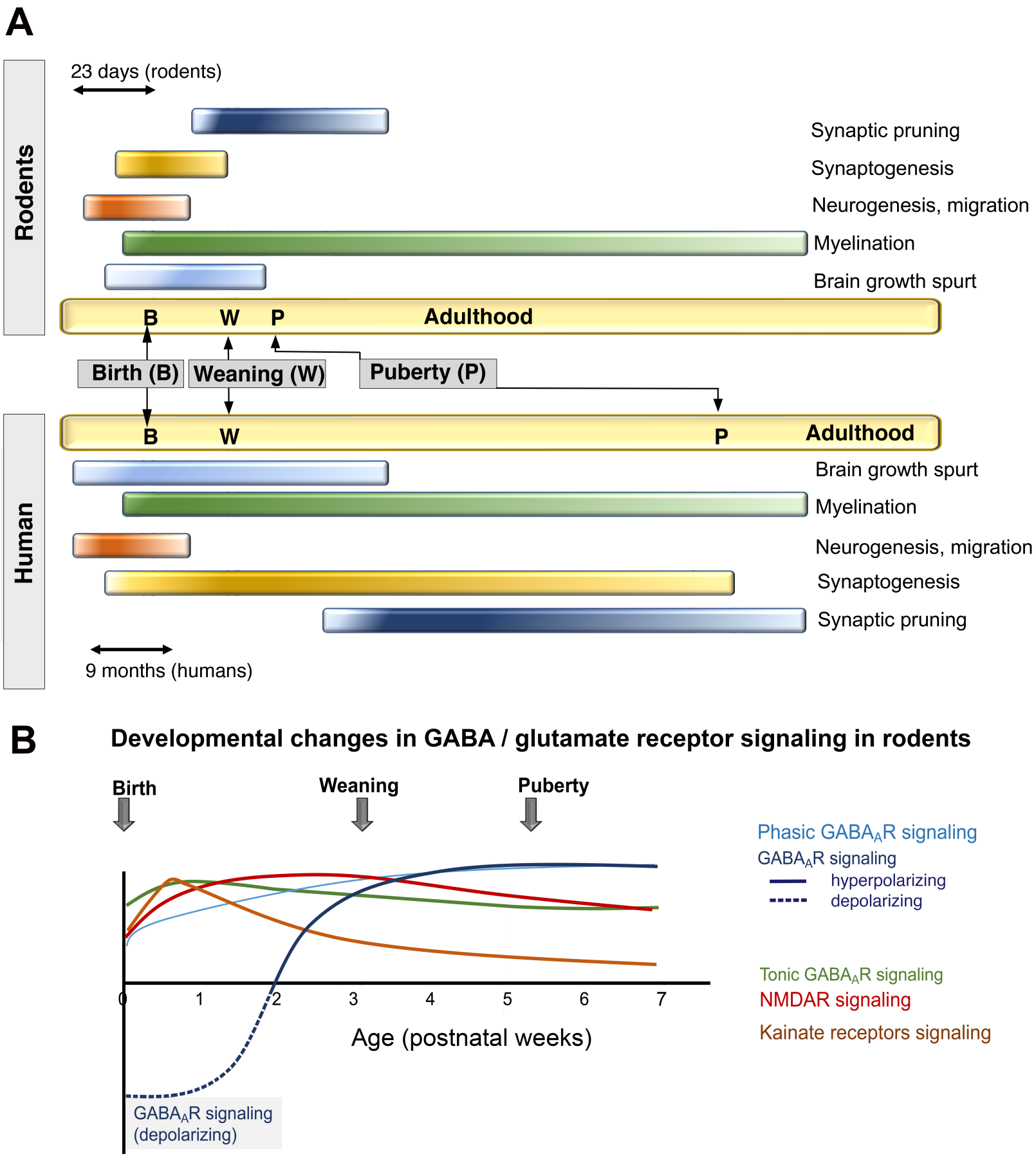
Spike and wave EEG

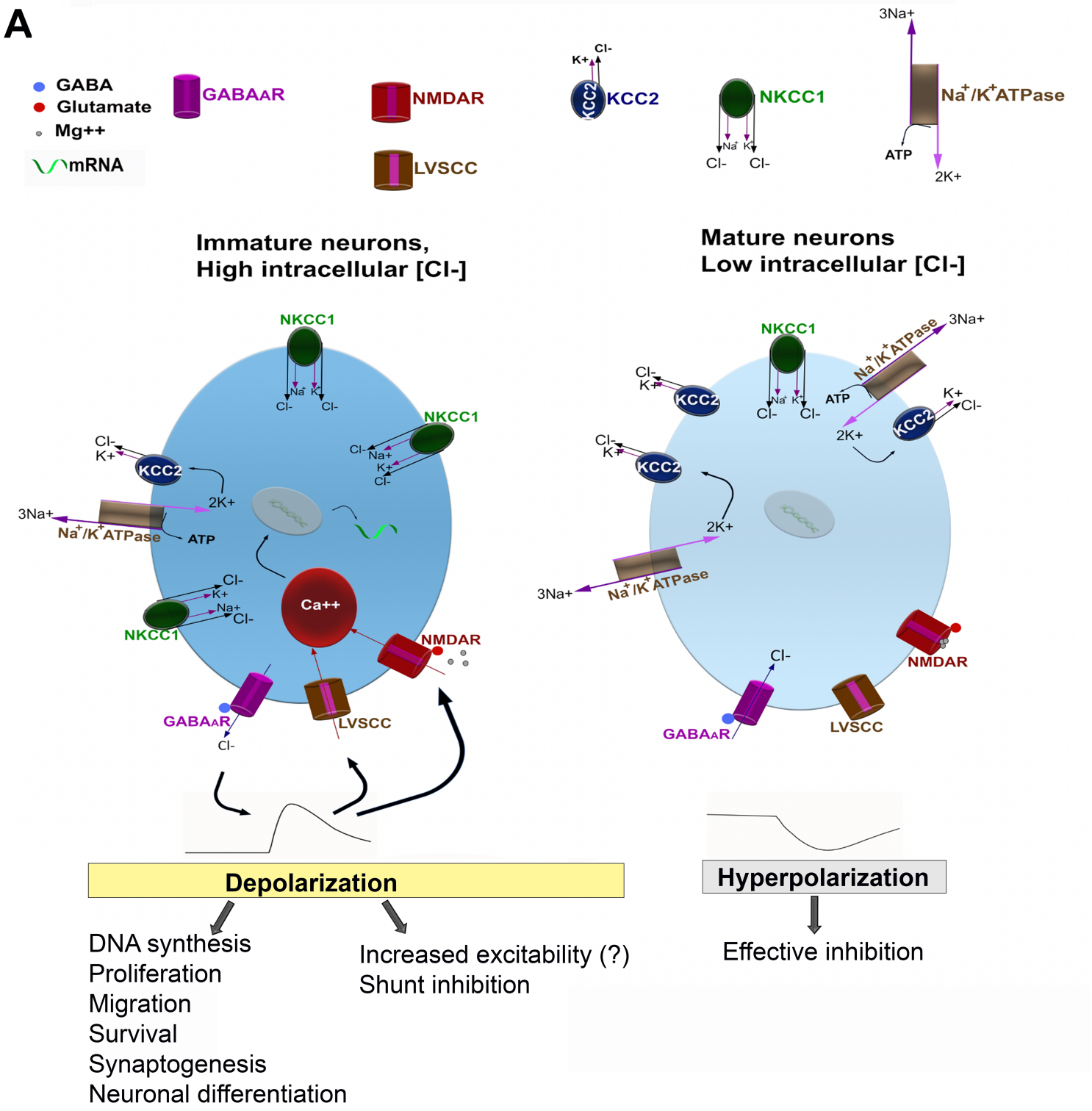
B

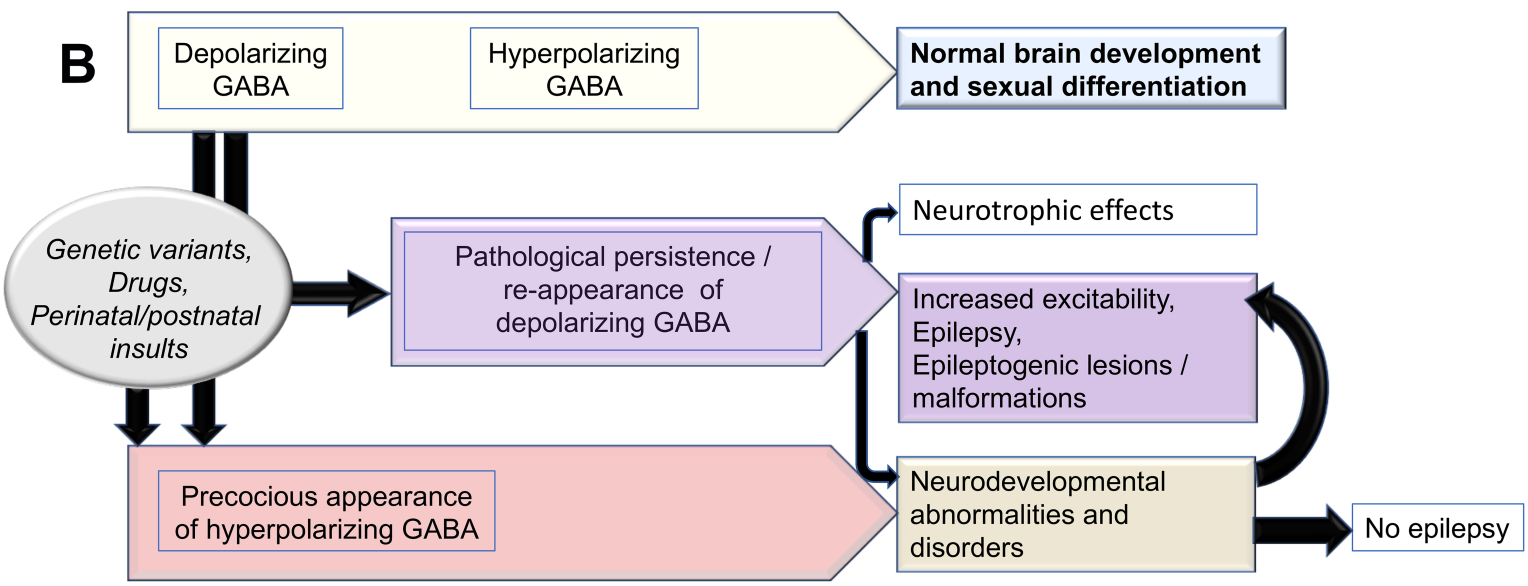


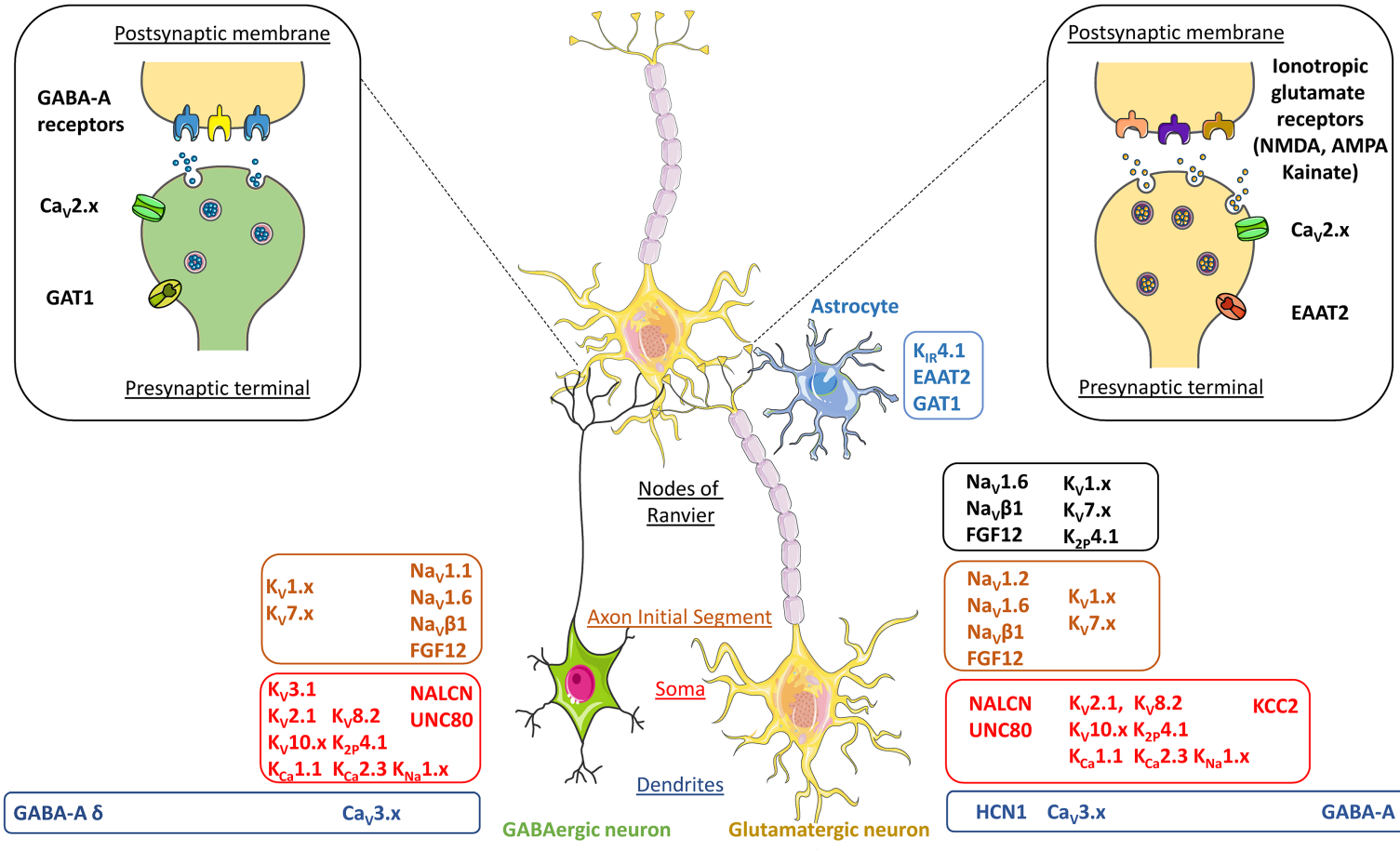


SECONDARY
GENERALIZATION


A
Rodents
23 days (rodents)
Synaptic pruning
Synaptogenesis
Neurogenesis, migration
Myelination
Brain growth spurt
B
W
P
Adulthood
Birth (B)
Weaning (W)
Puberty (P)
Human
Brain growth spurt
Myelination
Neurogenesis, migration
Synaptogenesis
Synaptic pruning
9 months (humans)
B
Developmental changes in GABA / glutamate receptor signaling in rodents
Birth
Weaning
Puberty
Phasic GABA$_A$R signaling
GABA$_A$R signaling
hyperpolarizing
depolarizing
Tonic GABA$_A$R signaling
NMDAR signaling
Kainate receptors signaling
0
1
2
3
4
5
6
7
Age (postnatal weeks)
GABA$_A$R signaling
(depolarizing)


A
GABA
Glutamate
Mg++
mRNA
GABAAR
NMDAR
LVSCC
KCC2
NKCC1
Na+/K+ATPase
Immature neurons,
High intracellular [Cl-]
Mature neurons
Low intracellular [Cl-]
Ca++
ATP
Depolarization
Hyperpolarization
DNA synthesis
Proliferation
Migration
Survival
Synaptogenesis
Neuronal differentiation
Increased excitability (?)
Shunt inhibition
Effective inhibition


B
Depolarizing GABA
Hyperpolarizing GABA
Normal brain development and sexual differentiation
Genetic variants, Drugs, Perinatal/postnatal insults
Pathological persistence / re-appearance of depolarizing GABA
Neurotrophic effects
Increased excitability, Epilepsy, Epileptogenic lesions / malformations
Precocious appearance of hyperpolarizing GABA
Neurodevelopmental abnormalities and disorders
No epilepsy

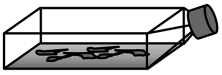

Postsynaptic membrane
GABA-A receptors
$Ca_V2.x$
GAT1
Presynaptic terminal
Postsynaptic membrane
Ionotropic glutamate receptors (NMDA, AMPA Kainate)
$Ca_V2.x$
EAAT2
Presynaptic terminal
Astrocyte
$K_{IR}4.1$
EAAT2
GAT1
Nodes of Ranvier
$Na_V1.6$ $K_V1.x$
$Na_V\beta1$ $K_V7.x$
FGF12 $K_{2P}4.1$
$K_V1.x$ $Na_V1.1$
$K_V7.x$ $Na_V1.6$
$Na_V\beta1$
FGF12
Axon Initial Segment
$Na_V1.2$ $K_V1.x$
$Na_V1.6$ $K_V7.x$
$Na_V\beta1$
FGF12
$K_V3.1$ NALCN
$K_V2.1$ $K_V8.2$ UNC80
$K_V10.x$ $K_{2P}4.1$
$K_{Ca}1.1$ $K_{Ca}2.3$ $K_{Na}1.x$
Soma
NALCN $K_V2.1$, $K_V8.2$ KCC2
UNC80 $K_V10.x$ $K_{2P}4.1$
$K_{Ca}1.1$ $K_{Ca}2.3$ $K_{Na}1.x$
Dendrites
GABA-A δ $Ca_V3.x$
GABAergic neuron
Glutamatergic neuron
HCN1 $Ca_V3.x$ GABA-A δ

Human brain

EPS, MRI, BEH

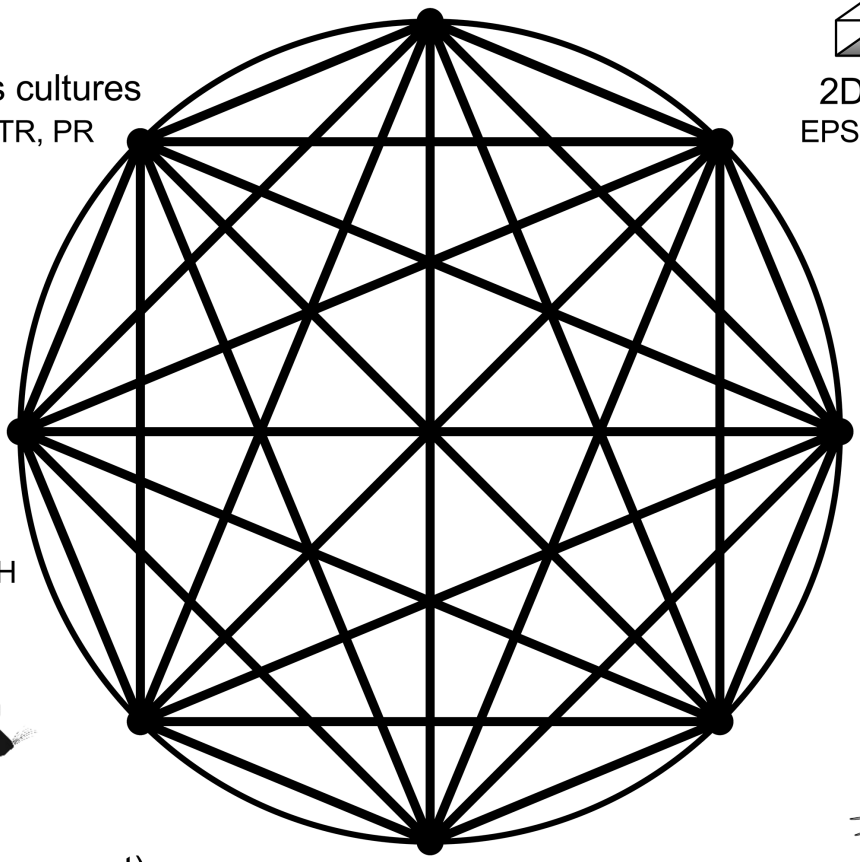
Human brain slices cultures

EPS, LI, IHC, ISH, TR, PR



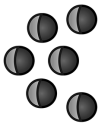
2D cell cultures

EPS, ICC, LI, TR, PR



Brain organoids

EPS, LI, IHC, ISH, TR, PR, BEH



Frog oocytes

EPS, ICC, LI, TR, PR

Rodent models (mouse, rat)

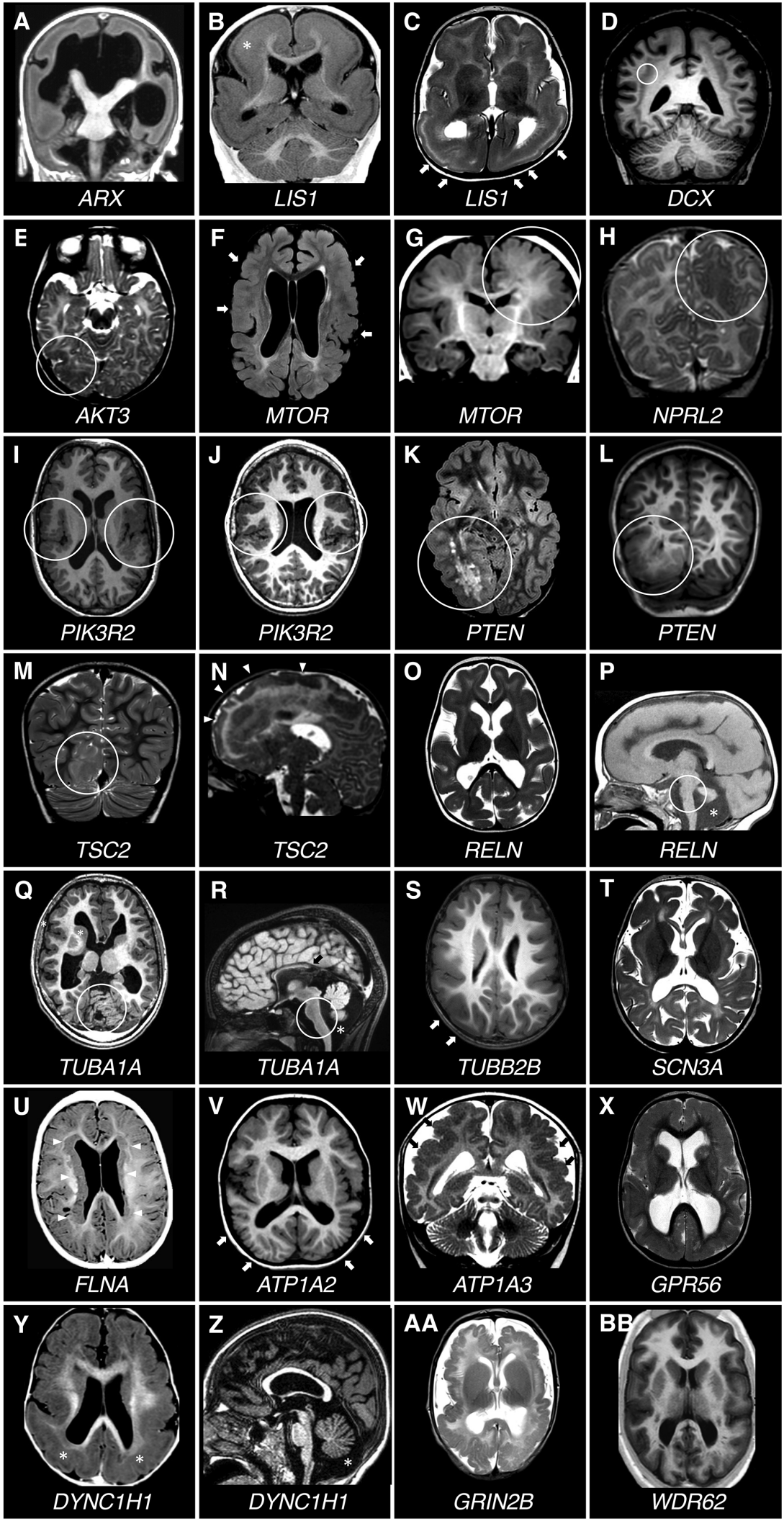
EPS, MRI, LI, IHC, ISH, TR, PR, BEH

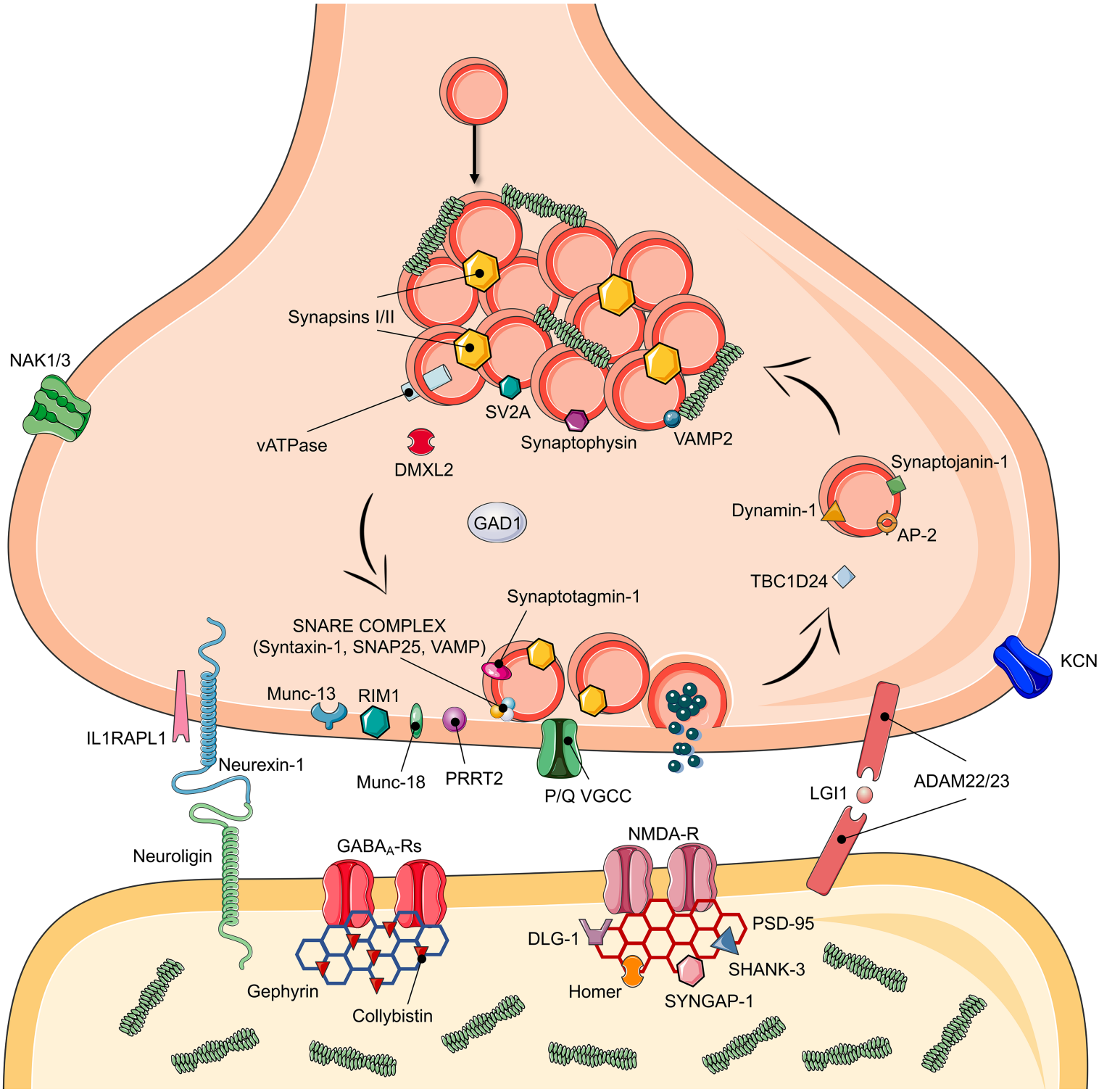
Fruit fly

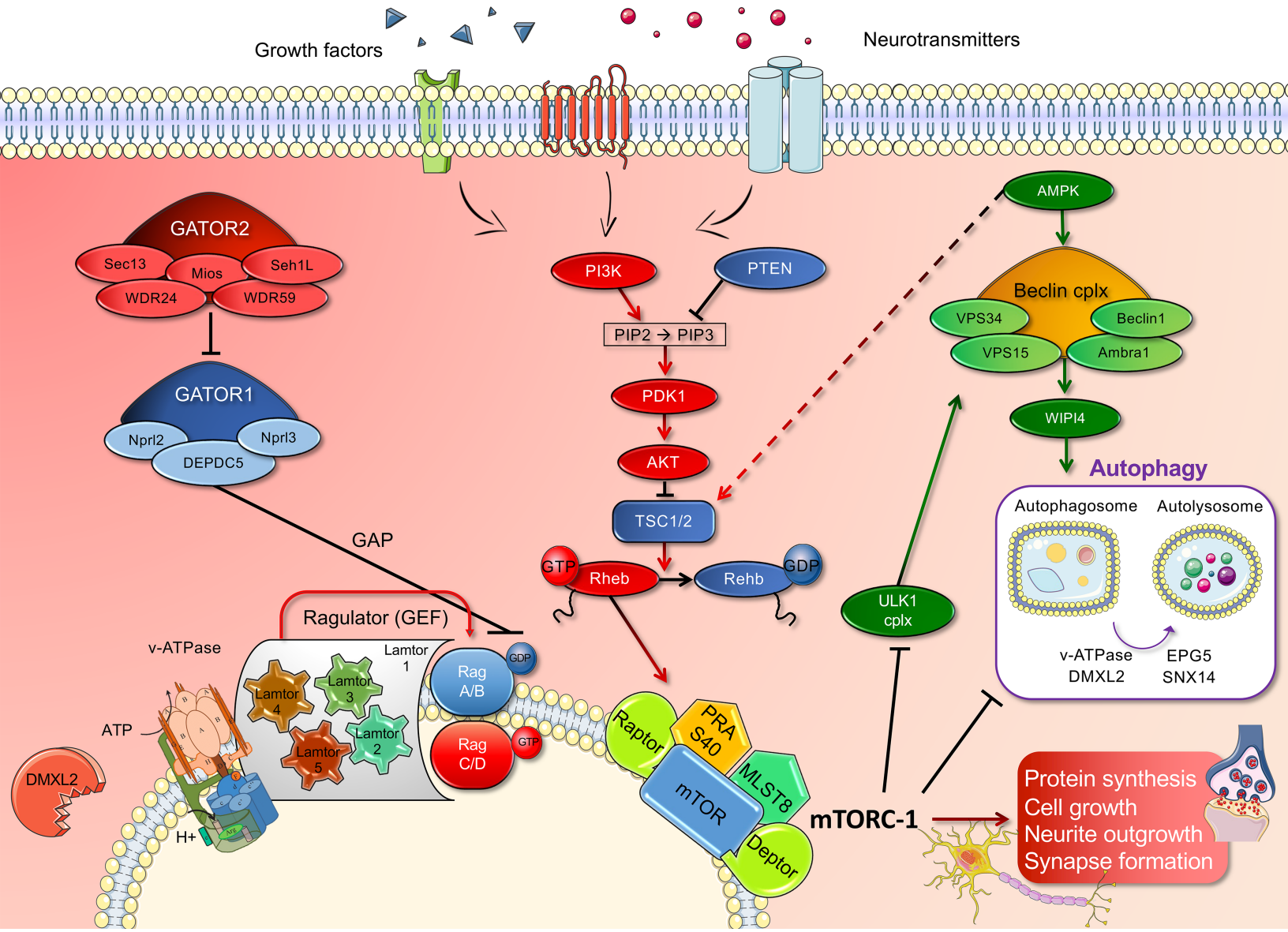
EPS, MRI, LI, IHC, ISH, TR, PR, BEH

Lower vertebrates (zebrafish, frog)

EPS, MRI, LI, IHC, ISH, TR, PR, BEH


A
ARX
B
LIS1
C
LIS1
D
DCX
E
AKT3
F
MTOR
G
MTOR
H
NPRL2
I
PIK3R2
J
PIK3R2
K
PTEN
L
PTEN
M
TSC2
N
TSC2
O
RELN
P
RELN
Q
TUBA1A
R
TUBA1A
S
TUBB2B
T
SCN3A
U
FLNA
V
ATP1A2
W
ATP1A3
X
GPR56
Y
DYNC1H1
Z
DYNC1H1
AA
GRIN2B
BB
WDR62


NAK1/3
Synapsins I/II
SV2A
vATPase
DMXL2
Synaptophysin
VAMP2
Synaptojanin-1
Dynamin-1
AP-2
GAD1
TBC1D24
Synaptotagmin-1
SNARE COMPLEX
(Syntaxin-1, SNAP25, VAMP)
KCN
Munc-13
RIM1
IL1RAPL1
Neurexin-1
Munc-18
PRRT2
P/Q VGCC
LGI1
ADAM22/23
Neuroligin
GABA$_A$-Rs
NMDA-R
DLG-1
PSD-95
SHANK-3
Homer
SYNGAP-1
Gephyrin
Collybistin


Growth factors
Neurotransmitters
GATOR2
Sec13
Mios
Seh1L
WDR24
WDR59
GATOR1
Nprl2
Nprl3
DEPDC5
GAP
PI3K
PTEN
PIP2 → PIP3
PDK1
AKT
TSC1/2
GTP
Rheb
Rehb
GDP
Ragulator (GEF)
v-ATPase
Lamtor 1
Lamtor 4
Lamtor 3
Lamtor 2
Lamtor 5
Rag A/B
Rag C/D
GDP
GTP
ATP
H+
DMXL2
Raptor
PRAS40
MLST8
mTOR
Deptor
mTORC-1
AMPK
Beclin cplx
VPS34
Beclin1
VPS15
Ambra1
WIPI4
Autophagy
Autophagosome
Autolysosome
v-ATPase
DMXL2
EPG5
SNX14
ULK1
cplx
Protein synthesis
Cell growth
Neurite outgrowth
Synapse formation