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Defects at the crossroads of GABAergic signaling in generalized genetic epilepsies

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Abstract

Seizure disorders are very common and affect 3% of the general population. The recurrent unprovoked seizures that are also called epilepsies are highly diverse as to both underlying genetic basis and clinic presentations. Recent genetic advances and sequencing technologies indicate that many epilepsies previously thought to be without known causes, or idiopathic generalized epilepsies (IGEs), are virtually genetic epilepsy as they are caused by genetic variations. IGEs are estimated to account for ~15-20% of all epilepsies. Initially IGEs were primarily considered channelopathies, because the first genetic defects identified in IGEs involved ion channel genes. However, new findings indicate that mutations in many non ion channel genes are also involved in addition to those in ion channel genes. Interestingly, mutations in many genes associated with epilepsy affect GABAergic signaling, a major biological pathway in epilepsy. Additionally, many antiepileptic drugs work via enhancing GABAergic signaling. Hence, the review will focus on the mutations that impair GABAergic signaling and selectively discuss the newly identified *STXBP1*, *PRRT2*, and *DNM1* in addition to those long-established epilepsy ion channel genes that also impair GABAergic signaling like *SCN1A* and $GABA_A$ receptor subunit genes. GABAergic signaling includes the pre- and post- synaptic mechanisms. Some mutations, such as *STXBP1, PRRT2, DNM1*, and *SCN1A*, impair GABAergic signaling mainly via pre-synaptic mechanisms while those mutations in $GABA_A$ receptor subunit genes impair GABAergic signaling via post-synaptic mechanisms. Nevertheless, these findings suggest impaired GABAergic signaling is a converging pathway of defects for many ion channel or non ion channel mutations associated with genetic epilepsies.

Keywords

Ion channels; non ion channels; mutations; GABAergic signaling; epilepsy; vesicles

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Introduction

Epilepsy is a common neurologic disorder, and the causes are highly heterogeneous. Genetic generalized epilepsies (GGEs) refer to epilepsy syndromes previously classified as idiopathic generalized epilepsies (IGEs) (Scheffer et al., 2017), which have been associated with variations in multiple genes based on recent genetic advances (Klassen et al., 2011). The incidence of epilepsy (recurrent unprovoked seizures) in children and adolescents ranges from 50 to 100/100,000 (Hauser, 1994). The general frequency of IGEs is estimated to be 15–20% of all epilepsies (Jallon and Latour, 2005). GGEs are a group of neurological disorders which is common in both the pediatric population and andadults (Hauser, 1994). GGEs include several different epilepsy syndromes that vary in clinical severity from relatively benign childhood absence epilepsy (CAE), which may remit with age, to more severe juvenile myoclonic epilepsy (JME), and generalized epilepsy with febrile seizures plus (GEFS+). A subpopulation of GGEs are associated with severe recurrent seizures and cognitive decline that have been referred to as epileptic encephalopathies, which are often refractory to existing treatments and have poor developmental outcome. Epileptic encephalopathies include severe myoclonic epilepsy in infancy (SMEI) or Dravet syndrome, West syndrome or infantile spasms, Ohtohara syndrome, and Lennox-Gastaut syndrome.

Many ion channel and non ion channel gene mutations have been identified in various epilepsies including epileptic encephalopathy (Merwick et al., 2012). The identified ion channel genes include both voltage gated and ligand gated ion channel gene mutations. The voltage gated ion channels affected by mutations include but are not limited to *SCN1A, SCN2A, SCN3A, SCN8A, SCN1B, KCNB1, KCNQ2*, *KCNQ3, Cav3.1, Cav3.2* and *Cav3.3.* As for the ligand gated ion channels, the genes include *ChRNA4* and *ChRNB2* as well as mutations in $GABA_A$ receptors including *GABRA1, GABRB1–3, GABRG2* and *GABRD* (Anderson et al., 2002;Macdonald et al., 2010). To date, most functional studies of epilepsy genetic mutations have been focused on ion channel genes. Mutation of ion channels that cause either a “gain of function” in excitatory neurotransmission or a “loss of function” in inhibitory neurotransmission could impair the balance of excitation and inhibition, leading to disinhibition and hyper-excitability in the brain.

In addition to mutations in ion channel genes, many mutations in non ion channel genes have also been associated with various kinds of epilepsies. This has changed our traditional view of epilepsy as channelopathies with defects in ion channels. Many non ion channel genes that are associated with epilepsy are still unfamiliar to the field of epilepsy research. The biological function of these genes or how these genes make the brain epileptic is not clear. These genes include but not limited to *PCDH19, CDKL5, STXBP1, STX1B, DNM1, PRRT2, CHD2, IQSE2, FOXG1, ALG3, RELN*, etc. There is no doubt that the list of genes associated with epilepsy is still growing. This suggests an urgent need for defining biological functions of these genes and their roles in epileptogenesis.

Impaired GABAergic signaling is a converging pathway of pathophysiology in genetic epilepsy

Whether the mutations are in ion channel genes or non ion channel genes, they are likely to cause defects in possible common converging pathways that are critical for seizure

generation. Among all the newly identified epilepsy mutations, ion channel genes continue to have the most frequent occurrence on the list. This suggests the prominent role of ion channels in the pathogenesis of epilepsy. Activation of neurotransmitter receptor ion channels at synapses promotes synapse plasticity during brain development. Consequently, impaired ion transport may affect neural excitability and brain development, resulting in epilepsy and other neurodevelopmental disorders. Further, synapse formation and normal function are essential in the signaling and the formation of neural networks. Genes related to synapse formation and function are also closely related to epilepsy and other neurodevelopmental disorders like autism and mental disability (Delahanty et al., 2011). Similarly, some non ion channel genes in the pathways of cell growth (Guo et al., 2013), transcriptional regulation, protein kinase modulation, cell metabolism, and cell-cell interaction may also participate in synapse formation and function while defects in these genes may lead to the genesis of epilepsy (Lubin, 2012;Scharfman and Brooks-Kayal, 2014).

GABAergic signaling is an established pathway for seizure generation. Not surprisingly, many mutations in both ion channel and non ion channel genes have been identified to impair GABAergic signaling. Here we will summarize different genes that impair GABAergic signaling and have been associated with epilepsy. The mechanisms by which these gene mutations impair GABAergic signaling include pre- and post-synaptic mechanisms. The epilepsy genes that impair GABAergic signaling via the pre-synaptic mechanisms include but are not limited to *STXBP1*, *STX1B*, *DNM1*, and *PRRT2*. These genes encode proteins that are involved in vesicle fusion machinery and vesicle release. The defects in the vesicle fusion machinery affect the pre-synaptic vesicle release. Failure or impaired release of key neurotransmitters would profoundly impair the corresponding neurotransmission and synaptic activity.

GABAergic neurotransmission

The cardinal aspects of GABAergic neurotransmission include pre-synaptic neurotransmitter gamma amino butyric acid (GABA) release and the $GABA_A$ receptor post-synaptic mechanisms (Figure 1). GABA is released by GABAergic interneurons that provide much of the inhibition in the cerebral cortex, hippocampus, striatum and amygdala. Impaired interneuron function has been established as an underlying cause for epilepsy via multiple preclinical animal models (Powell, 2013). Along the same line, much effort has been taken to rescue interneuron function to treating epilepsy. For example, it has been reported that GABA progenitor cells grafted into the adult epileptic brain attenuated seizures and comorbidities in mice (Hunt et al., 2013;Hunt and Baraban, 2015).

GABA

GABA is the major inhibitory neurotransmitter while glutamate is the major excitatory neurotransmitter in the brain. Both neurotransmitters work together to control many neuronal processes including the overall brain excitation. It has been established that glutamic acid decarboxylase (GAD) converts glutamate to GABA. There are two isoforms of GAD, GAD65 and GAD67 that synthesize GABA in the brain. After released from pre-

synaptic terminals, GABA is taken up by GABA transporters. These transporters are widely expressed in neuronal (mainly GAT-1) and glial (mainly GAT-3) cells throughout the brain. Inside the cell, GABA is degraded by GABA transaminase to succinic semialdehyde, and inhibition of this enzyme by the antiseizure drug (ASD) like vigabatrin increases GABAergic neurotransmission. Because of the critical role of GAD in synthesizing GABA, it has been proposed that GAD65 loss of function may preferentially decrease the pre-synaptic reserve pool of GABA and decrease tonic GABA inhibition, leading to increased seizure susceptibility (Kash et al., 1997). Although no human GAD mutations have been found to consistently cause epilepsy, mutations in co-factors that are necessary for GAD65 function have been linked with early life seizures, as occurs in pyridoxine-dependent epilepsy (Kure et al., 1998). GABA acts through fast chloride-permeable ionotropic $GABA_A$ receptors and also through slower metabotropic G-protein-coupled $GABA_B$ receptors. Since there is no mutation that has been identified in $GABA_B$ receptors associated with epilepsy up to date, this review will only focus on $GABA_A$ receptors.

$GABA_A$ receptors

$GABA_A$ receptors are the primary mediators of fast inhibitory synaptic transmission in the central nervous system and have been repeatedly documented to play a critical role in animal models of seizures (Banerjee et al., 1998;Cohen et al., 2003;Evans et al., 1994;Feng et al., 2001;Kapur and Macdonald, 1997;Karle et al., 1998;Kohling et al., 2000;Poulter et al., 1999). These inhibitory receptors are hetero-pentomeric protein complexes composed of multiple subunits that form ligand gated, anion-selective channels. $GABA_A$ receptors are modulated by barbiturates, benzodiazepines, zinc, ethanol, anesthetics, and neurosteroids. $GABA_A$ receptors are formed by the assembly of multiple subunit subtypes (α1-α6, β1-β3, γ1-γ3, δ, ε, π, θ, and ρ1-ρ3). These $GABA_A$ receptor subunits are each encoded by a different gene and form chloride ion channels when assembled in a complete receptor. In the brain, $GABA_A$ receptors most commonly contain two α subunits, two β subunits, and a γ or δ subunit. The most common $GABA_A$ receptor is the α1β2γ2 subtype, but multiple subtype combinations exist. They vary in different brain regions and cell types and during different times in development. Subunit composition of $GABA_A$ receptor plays a major role in determining the intrinsic properties of each channel, including affinity for GABA, kinetics, conductance, allosteric modulation, probability of channel opening, interaction with modulatory proteins, and subcellular distribution.

Antiseizure drugs (ASDs) that take effect via enhancing GABAergic signaling

Potentiation of inhibitory neurotransmission mediated by GABA remains a key mechanism of ASD action as many ASDs are designed to work via modulating $GABA_A$ receptors or enhance GABAergic signaling. Additionally, some ASDs work via other mechanisms like limitation of sustained repetitive neuronal firing via blockade of voltage-dependent sodium channels or blockade of glutamatergic excitatory neurotransmission (Meldrum, 1996;Taylor and Meldrum, 1995). Neurons that use GABA as their neurotransmitter represent only a small fraction of neurons in regions that are essential to epileptic activity (Houser, 2014), such as the neocortex, hippocampus and amygdala. Parvalbumin interneurons have been shown to be associated with epileptic activity (Ma and Prince, 2012;Rubinstein et al., 2015). These inhibitory connections are vital in restraining the natural tendency of recurrently

connected excitatory neurons to undergo the transition through positive feedback into synchronized epileptiform discharges.

$GABA_A$ receptors that contain α1–3, 5 subunits and γ2 subunits are sensitive to benzodiazepines and γ2 subunits are critical for clustering the receptors at synapses contributing to phasic inhibition (Alldred et al., 2005). Phasic inhibition refers to the effects of GABA released at GABAergic synapses that binds to post-synaptic receptors located at the synaptic cleft, in contrast to extra- or peri-synaptic receptors that are activated by ambient GABA, which is referred as tonic inhibition. Phasic inhibition is primarily related to increased conductance when chloride channels open and hyperpolarization of post-synaptic membrane potential when chloride influx occurs. By contrast, δ-subunit-containing $GABA_A$ receptors are not present in synapses but in extra- or peri-synaptic regions. $GABA_A$ receptors that contain δ subunit can be potentiated by neurosteroids (Bianchi and Macdonald, 2003). This feature has been proposed to treat epilepsy. For example, Ganaxolone, a synthetic analog of allopregnanolone, has been proven to be beneficial for refractory focal epilepsy and infantile spasms (Broomall et al., 2014;Goodkin and Kapur, 2009;Rogawski et al., 2013). A recent phase II study indicates Ganaxolone reduces partial-onset seizures frequency (Sperling et al., 2017).

It is known that drugs blocking $GABA_A$ receptors, such as bicuculline and pentylenetetrazol, can cause seizures. This effect has been widely used in experimental animal models to study epilepsy. Conversely, enhancement of $GABA_A$ receptor-mediated inhibition is an effective antiepileptic approach that remains as a key mechanism for epilepsy drug discovery. Indeed, the first effective epilepsy treatment, bromide, works via enhancing GABAergic signaling (Krall et al., 1978). It has been demonstrated that bromide enhances GABA-activated currents in cultured neurons (Suzuki et al., 1994). Many ASDs have been developed because of their effect on GABAergic signaling potentiation. These drugs, including benzodiazepines, phenobarbital, felbamate, and topiramate, enhance the function of $GABA_A$ receptors. These drugs also include vigabatrin, tiagabine, gapapentin and valproate. Tigabine increases the level of GABAby blocking GAT-1, and hence, is classified as a GABA reuptake inhibitor (Rekling et al., 1990). Vigabatrin inhibits GABA-transaminase and increases brain GABA content (Petroff and Rothman, 1998). Valproate and gabapentin increase GABA synthesis and turnover (Loscher, 1989); both valproate and gabapentin have a range of activities that overlaps with those of drugs that are known to interact with GABA systems. In summary, these ASDs enhance GABAergic signaling either by enhancing $GABA_A$ receptor function or increasing GABA level in the synaptic cleft.

Mutations in genes that impair GABAergic signaling via pre-synaptic mechanisms

There are several epilepsy genes that may impair GABAergic signaling via pre-synaptic mechanism. It is possible that many other epilepsy genes whose biologic function is currently unknown may also be involved in this mechanism. Below we will discuss the genes that may directly or indirectly impair GABAergic signaling via pre-synaptic mechanisms.

***SCN1A* mutations—**Mutations in *SCN1A* are one of the main causes of genetic epilepsy (Anderson et al., 2002;Oliva et al., 2012). Loss of function mutations in *SCN1A* account for 80% of the most severe kind of epilepsy, Dravet syndrome (Marini et al., 2011). Sodium channel mutations that are associated with accelerated recovery from inactivation and increased sodium channel activity (i.e., those that produce a gain of function) can lead to enhanced seizure susceptibility, as in the epilepsy syndrome GEFS+ (Spampanato et al., 2001). The missense mutations in *SCN1A* are generally associated with relatively milder epilepsy syndromes like GEFS+ (Escayg and Goldin, 2010) while the loss of function mutations of *SCN1A* are associated with more severe epilepsy syndromes like Dravet syndrome (Meisler and Kearney, 2005). Although there is still some controversy over the findings in human-patient-derived induced pluripotent stem cells in which increased sodium current in both bipolar- and pyramidal-shaped neurons was observed (Isom, 2014;Liu et al., 2013), it is generally believed that mutations in *SCN1A* impair GABAergic interneuron activity (Escayg and Goldin, 2010;Kalume et al., 2007;Kalume et al., 2013;Yu et al., 2006). Reduced firing of inhibitory neurons would affect GABA release. This is consistent with enhancing $GABA_A$ receptor function by clobazam or other analogs that attenuate the seizure severity and rescue related comorbidity like autistic traits in a *Scn1a* knockout mouse model (Han et al., 2012).

Syntaxin Binding Protein 1 (*STXBP1*) mutations

A mutation in *STXBP1*, a gene also known as *Munc18–1*, was initially discovered as a cause for Ohtahara Syndrome (Saitsu et al., 2008). Ever since, it has been associated with many other epilepsy syndromes in early childhood and became one of the most prominent genes for epileptic encephalopathy (Stamberger et al., 2016). STXBP1 is a main part of the synaptic fusion machinery, which includes syntaxin, synaptobrevin, and SNAP25—the three main components (Ma et al., 2013). By binding to syntaxin, STXBP1 protein orchestrates the assembly of the other components. Syntaxin enables vesicles to fuse with the plasma membrane. In cells, the so-called minimal fusion machinery provides the final “push” for the vesicle to fuse with the membrane through proteins that twist around each other and pull the vesicle close enough to fuse.

A *STXBP1* knockout mouse model has been developed. In *STXBP1* heterozygous knockout mice, the reduction of readily releasable vesicles was greater in GABAergic neurons than glutamatergic neurons (Toonen et al., 2006). This thus suggests the contribution of GABAergic signaling in epilepsy associated with *STXBP1* mutations. However, the biologic functional study of *STXBP1* is still very limited. It is reported that deletion of *Munc18–1* in mice results in widespread neurodegeneration that remains poorly characterized. It has been demonstrated that the early stages of spinal motor circuit formation—including motor neuron specification, axon growth and pathfinding, and mRNA expression—are unaffected in *Munc18–1*(−/−) mice. This indicates that the role of *STXBP1* in synaptic activity is dispensable for early nervous system development (Law et al., 2016). A study in human embryonic stem (ES) cells (Patzke et al., 2015) indicated that heterozygous *STXBP1* mutations lower the levels of Munc18–1 protein and its binding partner, the t-SNARE-protein Syntaxin-1, by approximately 30% and decrease spontaneous and evoked

neurotransmitter release by nearly 50%. This suggests that heterozygous *STXBP1* mutations cause early epileptic encephalopathy specifically through a pre-synaptic impairment.

DNM1* mutations—DNM1* is a GTPase and plays an important role in pinching off the vesicle from the plasma membrane. *De novo* mutations in *DNM1* are a cause of severe epileptic encephalopathy like infantile spasms (Allen et al., 2013). Two recent publications have characterized the functional consequences of *DNM1* mutations and find that the seizure phenotype is largely due to the deleterious effects of *DNM1* mutations in GABAergic interneurons, while behavioral locomotor phenotypes may be due to the effect of the mutation in pyramidal cells (Asinof et al., 2016;Asinof et al., 2015). The loss of DNM1 in inhibitory neurons resulted in early onset lethal seizures with age at death ranging from postnatal 15–27 days old.

The mouse model of *DNM1*, referred to as the *fitful* mouse, is a spontaneous mouse mutant that was eventually found to have point mutation in *DNM1* in the middle domain of the DNM1 protein. The mouse *DNM1* gene has an alternatively spliced exon, which means that two variants of *DNM1* are produced from a single gene. Previous studies (Asinof et al., 2015;Boumil et al., 2010) suggest that the homozygous *fitful* mouse best recapitulates the heterozygous situation in humans given this alternative exon. Homozygous *Dnm1 fitful* mice develop severe seizures, ataxia, and usually die before the age of 14 days, while heterozygous *DNM1* mice only have a mild epilepsy phenotype that starts at the age of 2–3 months.

The findings indicate that when the wild-type *DNM1* is deleted from GABAergic interneurons the mice develop an epilepsy phenotype. The affected neurons seem to be parvalbuminergic neurons, a subclass of GABAergic interneurons. Gene deletion in these cells alone is capable of producing the same epilepsy phenotype as the deletion in all cells. When expressed in other interneuron subsets, the epilepsy phenotype is milder. But when *DNM1* was deleted from glutamatergic neurons and not GABAergic neurons, the animals with deletions in glutamatergic neurons did not develop seizures. However, these animal show abnormal locomotor, exploratory, and repetitive behaviors, suggesting that the glutamatergic gene deletion may in part be responsible for the non-epilepsy phenotypes in humans, such as developmental delay and autism. It is likely that different neuronal cell types in *DNM1* encephalopathy may be responsible for different aspects of the disease. The effect of the mutation in GABAergic interneurons is responsible for the epilepsy phenotype (Asinof et al., 2015). In summary, this suggests selective deletion of *DNM1* in GABAergic neuron is sufficient for seizure generation. The *DNM1* mutations in patients with epileptic encephalopathies act in a dominant-negative manner which results in less efficient vesicle endocytosis. GABAergic interneurons may be particularly prone to disruptions of this function given their fast firing frequency.

***PRRT2* mutations—**Proline-rich transmembrane protein 2 (PRRT2 protein) is a pre-synaptic transmembrane protein and a key component of the calcium-dependent neurotransmitter release machinery (Valente et al., 2016). It is thought that PRRT2 protein interacts with the vesicle cycle in several ways. First, it may act as one component of the SNARE complex itself. Secondly, it is involved in vesicle recycling. Third, it may have a

role in regulating the pre-synaptic ion channels that trigger the vesicle release. Mutations in PRRT2 protein are associated with benign familial infantile seizures (BFIS), paroxysmal kinesogenic dyskinesia (PKD) (Chen et al., 2011), infantile convulsions with choreoathetosis (ICCA) (Heron et al., 2012;Scheffer et al., 2012), and other atypical phenotypes. It is believed that PRRT2 protein interacts with members of the SNARE complex, namely SNAP25. The SNARE complex is involved in synaptic vesicle fusion and forms the minimal fusion machinery that allows synaptic vesicles to fuse with the plasma membrane. Both vesicles and the pre-synaptic membrane are lipid bilayers that repel each other. The synapse uses torsion of the proteins in the SNARE complex to overcome this repulsion – basically, the vesicle is pulled so close to the membrane that it eventually fuses. The detailed role of PRRT2 protein in modulating this process is unknown.

The mouse model of *PRRT2* knockout recapitulates the neurological diseases associated with *PRRT2* mutations (Michetti et al., 2017). This suggests haploinsufficiency of *PRRT2* underlies the pathophysiology of PKD, ICCA, and seizures associated with *PRRT2* mutations (Michetti et al., 2017). Although normal at birth, *PRRT2* knockout mice display paroxysmal movements at the onset of locomotion that persist into adulthood. In addition, adult *PRRT2* knockout mice present abnormal motor behaviors characterized by wild running and jumping in response to audiogenic stimuli and reduced seizure threshold. Patch-clamp electrophysiology in hippocampal and cerebellar slices revealed specific effects in the cerebellum, where *PRRT2* is highly expressed, consisting of a higher excitatory strength at parallel fiber-Purkinje cell synapses during high frequency stimulation. The results show that the *PRRT2* knockout mouse reproduces the motor paroxysms present in the human patients carrying *PRRT2* mutations. Although a recent study indicates that *PRRT2* mutation results in a decrease in the frequency of vesicle release probability (Valente et al., 2016), it is unclear how *PRRT2* mutation differentially affects inhibitory vs excitatory neurons. It is likely that mutations in *PRRT2*, as well as other genes that affect pre-synaptic mechanisms as mentioned above, affects both excitatory and inhibitory neurons. However, it is the effect on GABA neurotransmitter release that influences the epilepsy phenotype.

Mutations in other genes involved in impairing GABAergic signaling via pre-synaptic mechanisms

Mutations in other less-studied genes may also impair GABAergic signaling via pre-synaptic mechanisms. Since the initial discovery of *STXBP1* in Ohtahara syndrome, several other genes coding for proteins in the pre-synaptic fusion machinery have been identified as genes for human epilepsies. In addition to those aforementioned mutations, *STX1B* and *SNAP25* have also been found to be mutated in patients with genetic epilepsies. The molecular defects of *STX1B* (Schubert et al., 2014) and *SNAP25* (Rohena et al., 2013) may also involve pre-synaptic vesicle release. This emerging picture of impaired pre-synaptic vesicle release suggests that disruption of the regular function of pre-synaptic proteins may results in epilepsy. This mechanism may be counterintuitive given that global impairment of neurotransmitter release should primarily affect the excitatory neurons given the prominent presentation of pyramidal neurons in the brain. However, the epilepsy phenotype may be more related to the impairment of neurotransmitter release in GABAergic interneurons

resulting from the mutations. Consequently, this would result in overall reduced inhibition of neurotransmission, leading to a brain state more tilted toward convulsion.

The mutations that impair GABAergic signaling via post-synaptic mechanisms

As mentioned earlier, the neurotransmitter GABA activates both $GABA_A$ and $GABA_B$ receptors. Although there are reports that an antibody against $GABA_B$ receptor mediates epilepsy opsoclonus-myoclonus syndrome and ataxia (Hoftberger et al., 2013;Kruer et al., 2014), there is no mutation identified in $GABA_B$ receptor subunit genes up to date. Thus, we will only focus on $GABA_A$ receptor mutations in this review article.

$GABA_A$ receptor subunits form a super family that contains 19 subunits. Mutations or variants in several $GABA_A$ subunits have been associated with epilepsies. These subunit genes include *GABRA1*, *GABRB1, GABRB2, GABRB3*, *GABRG2*, and *GABRD* (Ishii et al., 2017a;Johannesen et al., 2016;Kang and Macdonald, 2016;Macdonald et al., 2006;Moller et al., 2017a). Most of these mutations have autosomal dominant inheritance, and thus the patients are heterozygous for the mutation. The seizures and epilepsy syndromes resulting from mutations in these $GABA_A$ receptor subunit genes include multiple GE syndromes and vary in severities. These include pure febrile seizures (FS) (Audenaert et al., 2006) and epilepsy syndromes such as CAE (Tanaka et al., 2008), mixed afebrile and febrile seizures (CAE and FS and GEFS+ including Dravet syndrome), and afebrile seizures (Baulac et al., 2001;Dibbens et al., 2004;Harkin et al., 2002;Kananura et al., 2002;Sun et al., 2008;Wallace et al., 2001). The epilepsy mutations include missense and nonsense mutations, as well as insertion or deletion mutations resulting in frame shift mutations in coding regions, and mutations in noncoding regions.

Epilepsy phenotypic heterogeneity of $GABA_A$ receptor subunit mutations— There is a great phenotypic heterogeneity of epilepsy syndromes associated with $GABA_A$ receptor subunit gene mutations. For example, mutations in *GABRA1* have been associated with childhood absence epilepsy (Maljevic et al., 2005), juvenile myoclonic epilepsy (Cossette et al., 2002), and generalized tonic clonic seizures (Lachance-Touchette et al., 2011), as well as Dravet, Ohtahara, and West syndromes (Carvill et al., 2014;Kodera et al., 2016). Studies from mouse models indicate that deletion of *GABRA1* is sufficient to cause absence epilepsy (Arain et al., 2012). The knockin mice carrying *GABRA1(A322D)* displayed absence and myoclonic jerks (Arain et al., 2015). The functional consequence of *GABRA1* mutations that are associated with Dravet syndrome has not been characterized.

Mutations in *GABRG2* have been associated with a spectrum of seizures and generalized epilepsy syndromes. Phenotypes associated with both missense and nonsense mutations in *GABRG2* are variable ranging from mild childhood absence epilepsy and febrile seizures (Baulac et al., 2001;Wallace et al., 2001), to GEFS+ and epileptic encephalopathies like Dravet syndrome(Harkin et al., 2002;Kang and Macdonald, 2016;Shen et al., 2017). The basis for the more severe epilepsy phenotypes with *GABRG2* mutations are likely related to the extent of receptor function reduction and the metabolism of the mutant γ2 subunit protein (Kang et al., 2013). This notion is also supported by the comparison of two *Gabrg2* loss-of-function mutations in mouse models, which revealed that the mouse with production of the aggregation-prone mutant γ2 subunits had a more severe epilepsy phenotype than the

mouse that had simple *Gabrg2* haploinsufficiency without the mutant γ2 subunit protein produced (Warner et al., 2016). Protein structure modeling indicates that different mutant γ2 subunits have differing stabilities and interactions with their wild-type subunit binding partners because they adopt different conformations and have different surface hydrophobicities and different tendencies to dimerize. The mutant γ2 subunit associated with the most severe epilepsy phenotype is more likely to form dimers/oligomers than other γ2 mutants, and these oligomers are prone to form ring-like structures (Wang et al., 2016). However, to date, it is unknown if there is a predictable trend for how the type and location of mutation may correlate with disease severity.

Recently, many mutations in *GABRB3* have been associated with febrile seizures, absence seizures, autism, infantile spasm, and Lennox-Gaustaut syndrome (Moller et al., 2017b). Some patients are associated with uncharacterized seizures and mental disability (Hamdan et al., 2014). The detailed biological consequences resulting from these mutations are less clear. The functional assays of *GABRB3* mutations in an *in vitro* cell system indicate the mutations reduced receptor trafficking and gating, resulting in a reduced net channel function (Janve et al., 2016). Future study from an *in vivo* mouse model may shed more light on understanding the defect caused by *GABRB3* mutations.

Mutations in *GABRB2* and *GABRD* have also been associated with different epilepsy syndromes. The epilepsy phenotype of *GABRB2* mutations include early onset myoclonic encephalopathy or generalized tonic clonic seizures and atypical seizures with intellectual disability (Ishii et al., 2017b;Srivastava et al., 2014). The mutations in *GABRD* are associated with febrile seizures and juvenile myoclonic epilepsy (Dibbens et al., 2004;Feng et al., 2006).

Impaired trafficking is a major abnormality resulting from $GABA_A$ receptor subunit gene mutations—We have demonstrated that loss or impairment of subunit protein on the cell surface is the most common defect for all the missense, nonsense, and other premature termination codon (PTC)-generating $GABA_A$ receptor subunit gene mutations, although gating defects has also been identified in some mutations. The reduced cell surface expression could be accompanied by a reduction of total subunit protein or, in some cases, an increased amount of the mutant protein intracellularly. This seems counterintuitive but the mutant protein is not functional, thus explaining the pathophysiology of disease phenotype. We demonstrated that the mutant $GABA_A$ receptor subunits (eg. *GABRG2(R82Q), GABRG2(R136X), GABRG2(Q390X)*) are retained inside the ER, which is the location where the immature $GABA_A$ receptor subunit resides once synthesized. With glycosylation studies, we have identified all the mutant subunits that have arrested glycosylation. When coexpressed with the wild-type partnering subunits, the mutant subunits only adopt the ER glycosylation that is the core glycosylation for the immature subunits, while the wild-type subunits have mature glycosylation, suggesting subunit trafficking beyond the trans-Golgi to the cell surface. The mutant subunits with only core glycosylation are retained in the ER, suggesting that they are nonfunctional.

$GABA_A$ receptor mutations only cause loss or impaired function of the mutant subunit.

To date most insights into the functional defects are from studies in *GABRG2* subunits, although some studies have been carried out in other *GABR* gene mutations. There are some *GABRG2* mutations that may result in a simple loss of function, nearly a simple loss of, or impaired function of the subunit. For example, we have demonstrated that there was no dominant negative suppression by γ2(R136X) subunits on the wild-type partnering subunits like the α1 subunit (Johnston et al., 2014). These mutations often result in a mutant subunit protein that is readily degraded without much interference of the biogenesis and function of the remaining wild-type subunits. Alternatively, nonsense or PTC generating mutations could result in nonsense mediated decay (NMD) that eliminates the mutant allele at the mRNA level if the PTCs occur in an early exon and activate NMD. The functional consequence of these mutations would be similar to the *Gabrg2*$^{+/-}$ knockout condition, which may represent a simple haploinsufficiency condition. It is interesting that mutations in *GABRG2* are more likely to be associated with febrile seizures than other $GABA_A$ receptor subunits (Boillot et al., 2015).

$GABA_A$ receptor epilepsy mutations cause cellular toxicity in addition to the loss or impaired function of the mutant subunit—Some $GABA_A$ receptor mutations may cause severe dominant negative suppression of the wild-type $GABA_A$ receptors while some mutations only cause simple haploinsufficiency or mild dominant negative suppression of the wild-type subunits. We have extensively studied *GABRG2* nonsense mutations and identified the degradation rate of the mutant protein is likely the modifier of dominant negative suppression and epilepsy phenotype (Kang et al., 2013). Using the nonsense *GABRG2* mutations as example, despite loss of function for all the truncated subunits, we have demonstrated that R136X has no dominant negative effect on the remaining α1β2 subunits and Q390X has a strong dominant negative suppression of the wild-type subunits, while W429X has a mild dominant negative effect on the remaining α1β2 subunits (Kang et al., 2009;Kang et al., 2013). Thus the degree of dominant negative suppression of each mutant γ2 subunit varies, likely depending on the specific structural disturbance of each specific mutation (Wang et al., 2016).

The comparison of mouse models of *Gabrg2* knockout and *Gabrg2*$^{+/Q390X}$ knockin mice has validated the hypothesis from *in vitro* studies. *Gabrg2* knockout mice displayed infrequent absence epilepsy in a seizure prone genetic background or only hyper-anxiety (Crestani et al., 1999;Reid et al., 2013;Warner et al., 2016). By contrast, *Gabrg2*$^{+/Q390X}$ knockin mice displayed spontaneous seizures, multiple neuropsychiatric comorbidities, and sudden death, featuring the major presentations of Dravet syndrome (Kang et al., 2015). Consistently, data from both *in vitro* and *in vivo* models indicate a slight compensatory increase of wild-type subunits in the half gene dose or heterozygous *Gabrg2*$^{+/-}$ knockout condition but a reduction of wild-type subunits in the dominant negative mutant condition. In addition to impaired channel function, the mutations with dominant negative suppression may also cause neuronal injury or death because of the sustained production and accumulation of the mutant toxic protein. The mutant subunits with dominant negative suppression are likely to form protein aggregates and may disturb cellular homeostasis because of the sustained production of the mutant protein (Kang et al., 2015).

Conclusions—The findings from clinic patients and experimental animal models indicate impaired GABAergic signaling is a common converging pathway underlying multiple epilepsy syndromes associated with these ion channel or non ion channel mutations. This thus suggests that modulating GABAergic signaling remains as an essential therapeutic approach for genetic epilepsy. Although the diagnosis can be highly precise with genetic sequencing, it is unlikely that each specific epilepsy syndrome could have a specific drug developed with the capacity of current technology. It is critical to identify a few common converging pathways that could serve as therapeutic target for more than one epilepsy syndrome. In this scenario, enhancing GABAergic signaling would be an ideal—if not the best—choice for therapeutic target.

Traditionally, there are a few approaches to enhance GABAergic signaling, which include increasing GABA and enhancing $GABA_A$ receptor function. One approach to increase GABA levels is via modifying or inhibiting the activity of enzymes and transporters that alter the dynamics of GABA. The examples of ASDs that modify GABA dynamics include valproate, gabapentin, and vigabatrin, which increase cellular GABA by inhibiting GABA-transaminase. Another approach is to selectively increase GABA release. However, there is no ASD available for this specific action. Currently, one of the widely used ASDs, levetiracetam (Keppra), specifically binds to the synaptic vesicle protein SV2A and reduces excitatory neurotransmitter release during trains of high frequency activity. SV2A knockout mice display a severe seizure after the first week indicating that SV2A may regulate signaling cascades involved in seizure generation (Crowder et al., 1999). Keppra has also been reported to affect both glutamate and GABA release (Meehan et al., 2012) but the vesicular release machinery may act differentially in glutamatergic and GABAergic nerve terminals (Janz et al., 1999). In the future, more selective ASDs could be designed to specifically enhance GABA release or reduce glutamate release if the vesicular release mechanisms that differentiate pyramidal cells and interneurons are clearly elucidated.

In addition to traditional ASDs, promoting protein trafficking would be another reasonable approach to enhance GABAergic signaling. Although there is no data available, based on the findings from *GABRG2* mutations (Wang et al., 2016;Warner et al., 2016;Xia et al., 2016), promoting $GABA_A$ receptor trafficking could attenuate disease phenotype. Potential therapeutic approaches would include increasing wild-type and/or mutant $GABA_A$ receptor channel numbers and function, or decreasing the disturbance of the cellular signaling by the presence of the mutant $GABA_A$ receptor subunit protein. The drug, whether via direct modulation of the receptor function or increasing receptor trafficking, should be effective in compensating the lost or impaired $GABA_A$ channel function. Thus, a combined therapeutic strategy to enhance the wild-type $GABA_A$ receptor channel function and eliminate production of mutant protein or promoting protein homeostasis might be beneficial.

Rapid advances in gene editing technology like CRISPR/Cas9 have brought new hope for the treatment of genetic diseases including epilepsies. For example, a recent report on using CRISPR/Cas9-mediated gene editing has successfully ameliorated neurotoxicity and alleviated disease phenotype in a mouse model of Huntington's disease (Yang et al., 2017). Given that CRISPR/Cas9 can permanently eliminate the expression of targeted genes, use of this approach should be able to remove the mutant allele, thus preventing the production of

the mutant protein and attenuating disease phenotype, especially in the condition with a dominant negative mutation like *GABRG2(Q390X)*.

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Abbreviations:

IGEs	idiopathic generalized epilepsies
GGE	genetic generalized epilepsies
CAE	childhood absence epilepsy
JME	juvenile myoclonic epilepsy
ASDs	antiseizure drugs

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Highlights

1. Impaired GABAergic signaling is a converging pathway for epilepsies associated with mutations in several unrelated genes.
2. GABAergic signaling could be impaired via pre- and post-synaptic mechanisms.
3. Mutations in some genes like *STXBP1, DNM1, PRRT2* and *SCN1A* may impair GABA release via pre-synaptic mechanisms.
4. Mutations in some genes like *GABRA1, GABRG2, GABRB2* and *GABRB3* impair GABAergic signaling via post-synaptic mechanisms.
5. Modulating GABAergic signaling remains an essential therapeutic approach for genetic epilepsies.

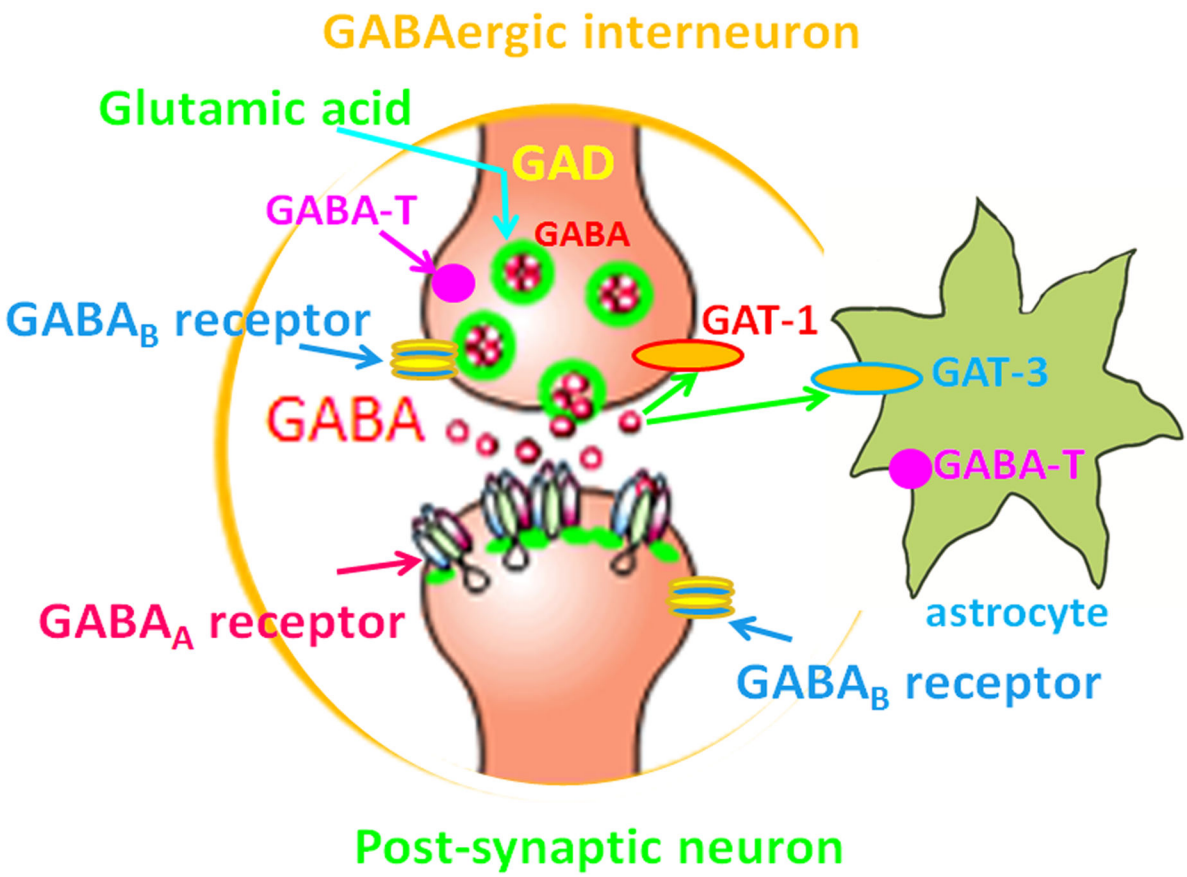

Figure 1.

GABA signaling. In GABAergic interneurons, the neurotransmitter GABA is synthesized from glutamic acid, the principal excitatory neurotransmitter via glutamic acid decarboxylase (GAD). GABA is catabolized by GABA transaminase (GABA-T) which is a membrane bound enzyme expressed by neurons and glia. GABA is released from vesicles in pre-synaptic terminals and activates GABA receptors which include $GABA_A$ receptors and $GABA_B$ receptors. $GABA_A$ receptors hyperpolarize neurons via Cl^- influx. The released GABA is taken up by GABA transporters (GAT-1 and GAT-3) back into pre-synaptic compartments of neurons or into astrocytes.

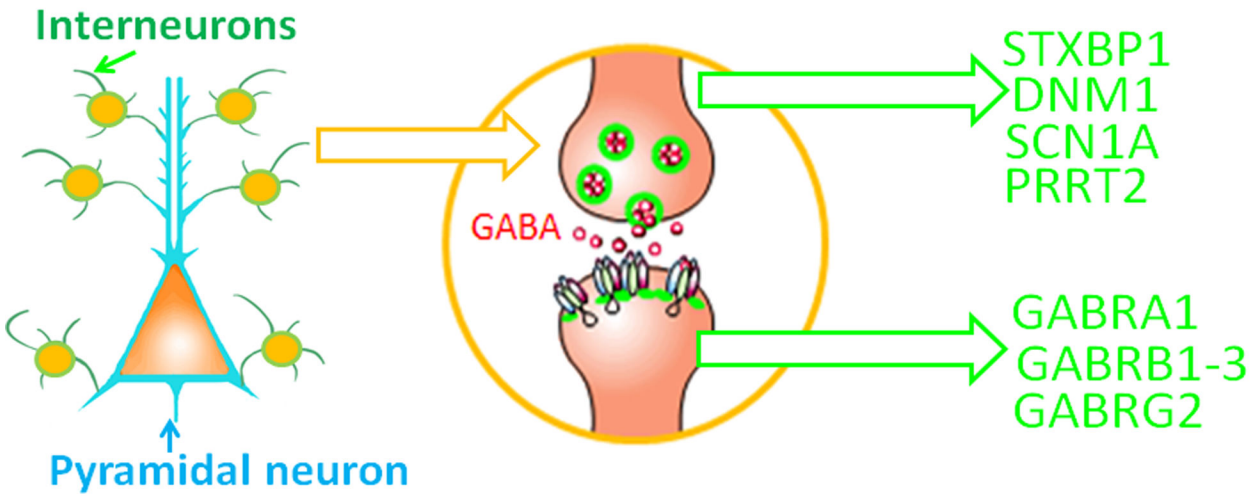

Figure 2.

Mutations via impairing both pre-synaptic GABA neurotransmitter release and post-synaptic $GABA_A$ receptor expression and function can affect GABAergic signaling. Synaptic transmission relies on the availability of the neurotransmitter; the release of the neurotransmitter by exocytosis and the binding of the normal functional postsynaptic receptor by the neurotransmitter. (A). Interneurons are the main source of cortical modulation over glutamatergic pyramidal cells (PCs). GABA-releasing interneurons as classified by a complex combination of morphological, connectivity, and intrinsic electrophysiological properties and molecular content are critical for cortical inhibition. (B). Mutations associated with epilepsy could impair both the proteins involving in pre-synaptic GABA release and post-synaptic $GABA_A$ receptor function.

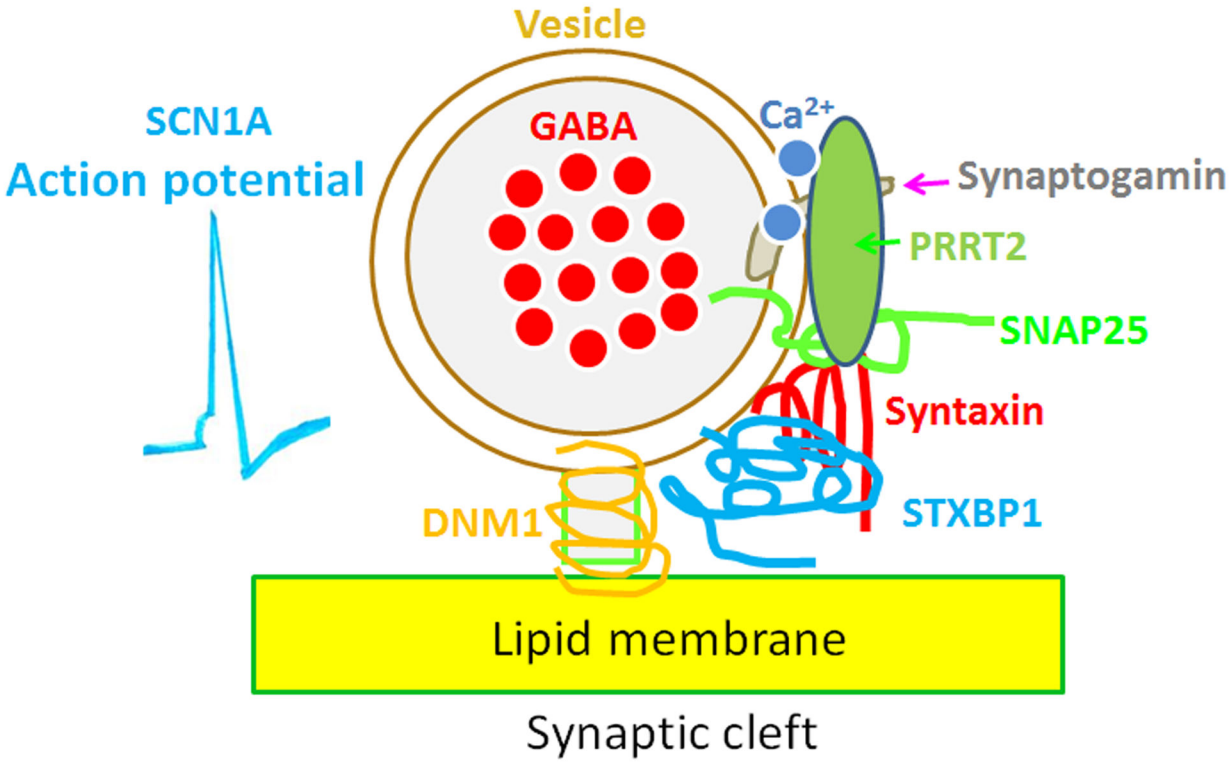

Figure 3.

Diverse defects caused by mutations in different genes impair GABA neurotransmitter release. In a given neuron, opening of sodium channels encoded by *SCN1A* and influx of Na + cause neuronal firing in which sodium channels are responsible for the rising phase of action potentials. Calcium enters the axon terminal during an action potential, causing release of the neurotransmitter into the synaptic cleft. Synaptogamin acts as a calcium sensor which binds calcium and activates vesicle fusion. Gene mutations that encode proteins involved in the process of vesicle release include but are not limited to *PRRT2*, *SNAP25*, *syntaxin*, *STXBP1* and *DNM1*. Although the biological function of each gene still requires further study, it has been proposed that these proteins are essential for making up the complicated vesicle release machinery for vesicle docking, fusion and exocytosis.

Table 1.

EE genes affecting GABAergic signaling and their postulated molecular defects.

GENE	LOCUS	pre- or post-synaptic mechanism	POSTULATED MECHANISMS	PHENOTYPES	REFERENCES (for mechanisms)
SCN1A	2q24	pre-synaptic	impaired interneuron activity	GEFS+, DS	Yu et al, 2006
PRRT2	16p11.2	pre-synaptic	impaired neurotransmitter release?	IS,EE, PKD	Valente et al, 2016
STXBP1	9q34.11	pre-synaptic	impaired neurotransmitter release?	GEFS+, DS	Patzke et al., 2015
STX1B	16p11.2	pre-synaptic	impaired neurotransmitter release?	FS, epilepsy	Schubert et al, 2014
SNAP25	20p12.2	pre-synaptic	impaired neurotransmitter release?	epilepsy and ID	Rohena et al., 2013
DNM1	9q34.11	pre-synaptic	impaired neurotransmitter release?	IS, LGS, EE	Asinof et al 2015
GABRA1	5q34	post-synaptic	NMD, ERAD	CAE, JME, EE, DS	Kang et al, 2009
GABRB2	5q34	post-synaptic	ERAD, reduced surface expression	EME	Ishii et al, 2016
GABRB3	15q12	post-synaptic	reduced surface expression, gating defect	CAE, IS, LGS, DS	Janve et al, 2016
GABRG2	5q34	post-synaptic	NMD, ERAD,gating defect, neuronal injury	FS, GEFS+,DS,EE	Kang et al, 2016

DS=Dravet syndrome, IS=infantile spasm, EE=epileptic encephalopathy

PKD=paroxysmal kinesigenic dyskinesia, ID=intellectual disability

CAE=childhood absence epilepsy, JME=Juvenile myoclonic epilepsy

EME=early myoclonic encephalopathy, FS=febrile seizures

LGS=Lennox Gaustaut syndrome

GEFS+=generalized epilepsy with febrile seizure plus