Chapter

Ion Channels in Epilepsy: Blasting Fuse for Neuronal Hyperexcitability

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Abstract

Voltage-gated ion channels (VGICs), extensively distributed in the central nervous system (CNS), are responsible for the generation as well as modulation of neuroexcitability and considered as vital players in the pathogenesis of human epilepsy, with regulating the shape and duration of action potentials (APs). For instance, genetic alterations or abnormal expression of voltage-gated sodium channels (VGSCs), Kv channels, and voltage-gated calcium channels (VGCCs) are proved to be associated with epileptogenesis. This chapter aims to highlight recent discoveries on the mutations in VGIC genes and dysfunction of VGICs in epilepsy, especially focusing on the pathophysiological and pharmacological properties. Understanding the role of epilepsy-associated VGICs might not only contribute to clarify the mechanism of epileptogenesis and genetic modifiers but also provide potential targets for the precise treatment of epilepsy.

Keywords: ion channels, VGSCs, Kv channels, Cav channels, TRPs, mutation, epilepsy

1. Introduction

Epilepsy is one of the chronic brain disorders characterized by recurrent seizures due to abnormal excessive electrical discharges of cerebral neurons [1]. It is believed that genetic factors play a crucial role in the etiopathogenesis of epilepsy. So far ~1000 genes have been proved to be associated with epilepsy, among which genes encoding VGIC predominate [2].

VGICs are pore-forming membrane proteins. Their functions include establishing APs and maintaining homeostasis by gating the ionic flow traversing the cell membrane, managing the ionic flow across cells and regulating Ca^{2+} signal transduction, which are essential to the neuroexcitability, so VGICs are potentially involved in epileptogenesis [2]. The association of VGIC genes and epilepsy might provide insights into the etiopathogenesis underlying epilepsy. Pathophysiological studies illuminated that two key defects are (i) a neuronal disinhibition induced by loss-of-function of VGIC gene expressed specifically in inhibitory interneurons (for example, Nav1.1 and P/Q VGCCs) or (ii) dysfunction of axon initial segments, the neuronal structure in which APs are generated and many VGICs (such as Nav1.2 and Kv7) are mainly localized (**Figure 1**). Moreover, clinically originated studies identified novel genes, defined their neuronal functions, and sometimes established novel physiological principles [2].

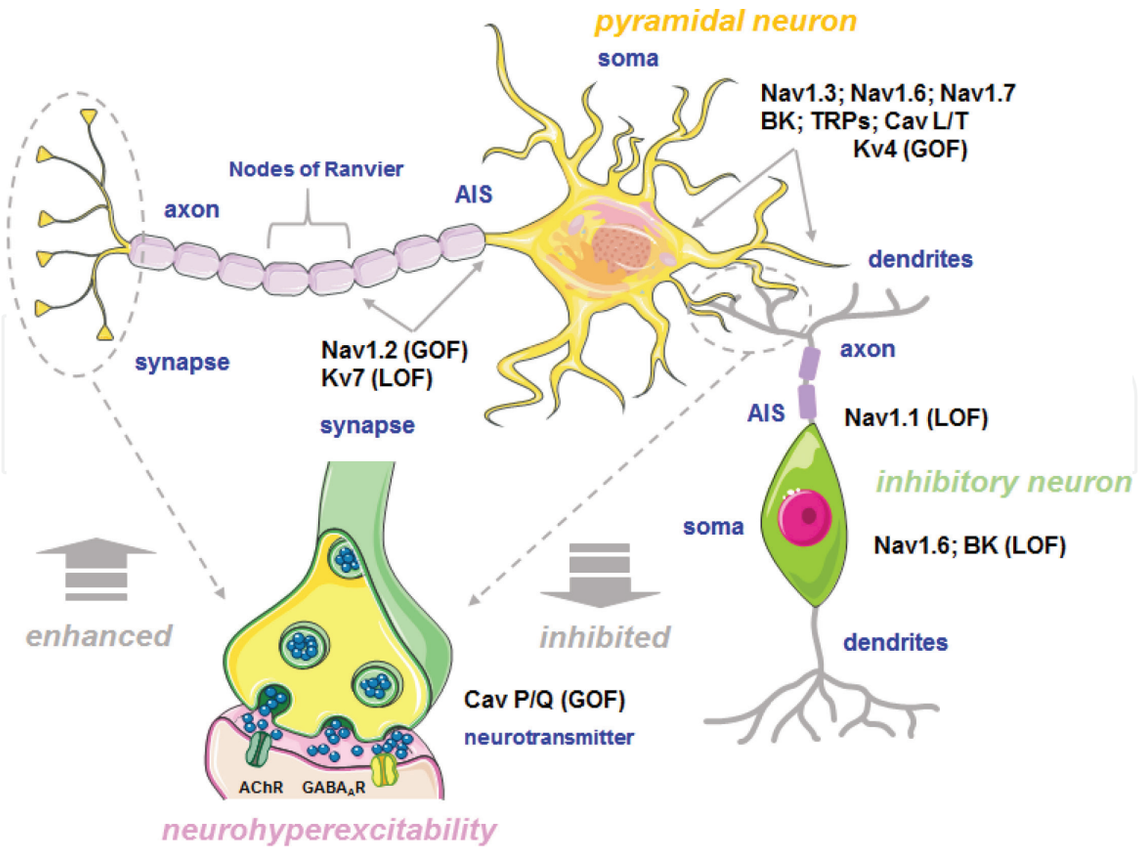

Figure 1.
Neuronal localization of some relevant voltage-gated ion channels. A schematic view of an excitatory pyramidal (orange), an inhibitory (green) neuron, and their synaptic connections is shown. Distinctive intracellular compartments are targeted by different populations of VGICs. Examples of which as mentioned in this chapter are shown here: in the somatodendritic compartment, Nav, Cav (L- and T-type), TRP, BK, and Kv channels; at axon initial segments (AIS) and nodes of Ranvier in pyramidal neurons, Nav1.2, Kv7 channels; at AIS of inhibitory neurons, Nav1.1; in the somatodendritic compartment of inhibitory neurons, BK and Nav1.6; in the presynaptic terminals, Cav P/Q type. GOF represents the gain-of-function mutation of VGICs-induced human epilepsy. LOF represents the loss-of-function mutation of VGICs.

In this chapter, we summarize the epilepsy-associated VGIC genes, the mutations, corresponding phenotypes, and functional changes, aiming to provide clues for evaluating the relationship between VGIC genes and epileptogenesis.

2. Voltage-gated sodium channels

VGSCs play a critical role in the generation and propagation of APs in neurons, genetic alterations in VGSC genes are considered to be associated with epileptogenesis. Mammalian VGSC is composed of a large pseudotetrameric pore-forming α subunit with a molecular weight of 260 KDa, and one or more auxiliary β subunits (30–40 KDa) [3–5] (**Figure 2**). Nine subtypes of VGSC α subunits have been found in humans, including Nav1.1-Nav1.9, encoded by the genes SCN1A-SCN5A, SCN8A-SCN11A, respectively.

2.1 Nav1.1

Nav1.1 is mainly distributed in the inhibitory GABAergic neurons of cerebellum and hippocampus. The Nav1.1 gene SCN1A is the clinically most relevant SCN gene for epilepsy. More than 1200 mutants have been identified to be associated with epilepsy; most of them are febrile seizures [6]. M145T mutation, a well-conserved amino acid in the first transmembrane segment of domain I of the

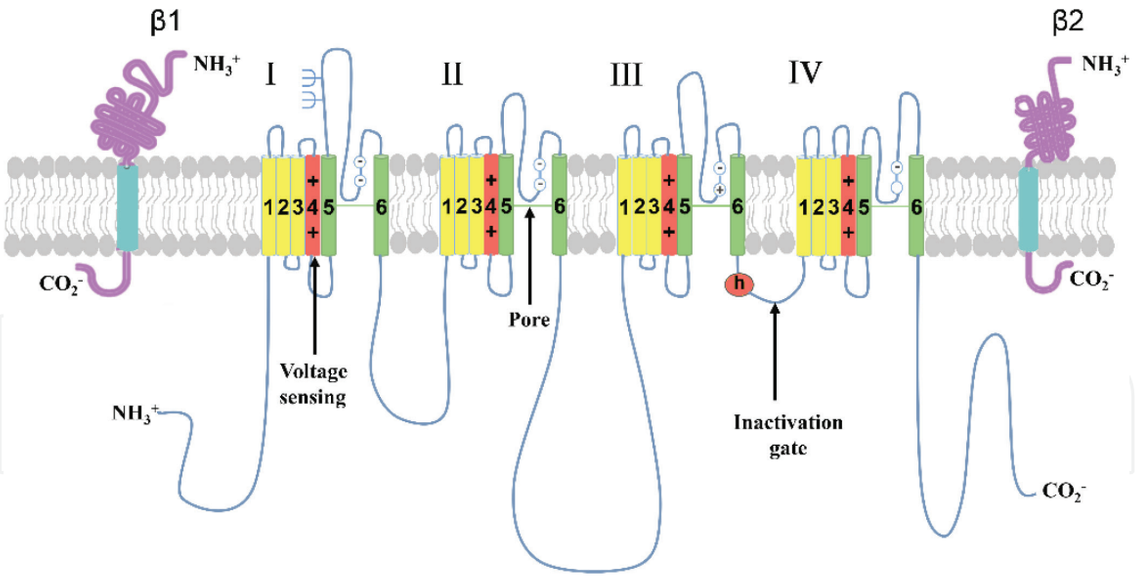

Figure 2.
Structure of voltage-gated sodium channels. Schematic representation of VGSC subunits. The α subunit of the VGSC is illustrated together with β1 and β2 subunits; extracellular domains of the β subunits are shown as immunoglobulin-like folds, interacting with the loops in α subunits. Roman numerals indicate the domains of the α subunit; segments 5 and 6 (shown in green) are the pore-lining segments, and S4 helices (red) make up the voltage sensors. The red circle in the intracellular loop of domains III and IV indicates the inactivation gate IFM motif; Ψ, probable N-linked glycosylation site. The circles in reentrant loops in each domain represent the residues that form the ion selectivity filter.

Nav1.1 α-subunit, caused a reduction in peak sodium currents and a positive shift in the voltage dependence of activation [7], which provided the first evidence that the mild loss-of-function mutations in Nav1.1 may cause a significant portion of febrile seizures. Complete loss-of-function mutations in Nav1.1 cause severe myoclonic epilepsy of infancy (SMEI or Dravet's syndrome), which includes severe, intractable epilepsy and comorbidities of ataxia and cognitive impairment. Besides, homozygous null Nav1.1$^{-/-}$ mice developed ataxia and died on half a month of postnatal and did not change the voltage-dependent activity of VGSCs in hippocampal neurons. However, heterozygous Nav1.1$^{+/-}$ mice exhibited spontaneous seizures and sporadic deaths after 3 weeks, and the sodium current density was substantially reduced in inhibitory interneurons, except in excitatory pyramidal neurons [8]. So loss-of-function mutations in Nav1.1 can severely impair sodium currents and AP firing in hippocampal GABAergic inhibitory neurons. The functional downregulation in inhibitory neurons might cause the hyperexcitability of dentate granule or pyramidal neurons, which could lead to epilepsy in patients with SMEI. Experiments in mice have demonstrated that haploinsufficiency of Nav1.1 channels is sufficient to allow induction of seizures by elevated body temperature, supporting that haploinsufficiency of SCN1A is pathogenic in human SMEI which has striking temperature and age dependence of onset and progression of epilepsy [9]. What is more, SCN1A mutations were mostly missense mutations in GEFS+ patients, which are typically well controlled by treatment with antiepileptic drugs and no cognitive impairment is observed. The R1648H channels showed the reduced function in both excitatory and inhibitory neurons although the biophysical mechanisms were different, reducing peak sodium currents and enhancing slow inactivation in inhibitory neurons versus negatively shifted voltage dependence of fast inactivation in excitatory neurons [10]. The similar conclusion had been drawn when the R1648H mutation has been inserted into the mouse genome under the native promoter [11]. In light of these results, GEFS+ and SMEI may be caused by a continuum of mutational effects that selectively impair firing of GABAergic inhibitory neurons, which lead to increase in the excitability of the neural network [12].

2.2 Nav1.2

The mutation of the Nav1.2 gene SCN2A is associated with various epilepsies, such as benign familial neonatal seizures (BFNIS), hereditary epilepsy with febrile seizures plus (GEFS+), Dravet's syndrome (DS), and other stubborn childhood epilepsy encephalopathy. Nav1.2 subunit is mainly distributed in the axon-initiating segment (AIS) and node of Ranvier. SCN2A mutations cause changes in VGSC function and expression and result in abnormal neuronal discharge. Because Nav1.2 plays an important role in the AIS area during the development, it is more common for infants to show SCN2A mutant-induced epilepsy encephalopathy [13]. BFNIS is the most common phenotype caused by gain-of-function missense mutations in SCN2A [14]. Up to now, at least 10 SCN2A mutations associated with BFNIS have been identified. SCN2A mutations are also found to result in the reduced expression of Nav1.2 on the surface of neurons [15]. Therefore, SCN2A mutants will lead to the decrease of sodium current density at node of Ranvier and AIS, seriously affecting the excitability of neurons [16]. For missense mutation of SCN2A, p.Tyr1589Cys causes a depolarizing shift of steady-state inactivation, increased persistent Na^+ current, a slowing of fast inactivation, and an acceleration of its recovery, which contribute to neuronal hyperexcitability and familial epilepsy [17]. Due to the SCN2A mutation, early infantile epileptic encephalopathy (EIEE) patients with burst suppression and tonic-clonic migrating partial seizures showed a specific dose-dependent efficacy of VGSC blockers [18]. It is mainly caused by the dysfunction of VGSC [19]. By replacing neonatal Nav1.2 with adult Nav1.2 in mice, it has been suggested that neonatal Nav1.2 reduced neuronal excitability and had a significant impact on seizure susceptibility and behavior.

2.3 Nav1.3

The SCN3A gene, clustered on human chromosome 2q24, encodes the Nav1.3 subtype [20], which is usually located in the soma of neurons. It is important in the integration of synaptic signals, determination of the depolarization threshold, and AP transmission [21]. In contrast to the rodent gene which is transiently expressed during development, human SCN3A is widely expressed in adult brain [22]. The first epilepsy-associated mutation (K354Q) in SCN3A was found in 2008. K354Q mutation decreased inactivation rate and increased I_{NaP} [23]. The mutation is not sensitive to antiepilepsy drug carbamazepine or oxcarbazepine. K354Q mutation causes neuronal abnormal spontaneous discharge and membrane potential paroxysmal depolarization [24]. In 2014, four more missense variants were identified in SCN3A, which are R357Q, D766N, E1111K, and M1323V [25]. Compared to wild-type channels, R357Q caused smaller currents, slower activation, and depolarized voltage dependences of activation and inactivation. The E1111K mutation evoked a significantly greater level of persistent sodium current. All four mutants increase current activation in response to depolarizing voltage ramps. These findings support for a contribution of Nav 1.3 to childhood epilepsy. Recently, a novel SCN3A variant (L247P) was identified by whole exome sequencing of a child with focal epilepsy, developmental delay, and autonomic nervous system dysfunction. Voltage clamp analysis showed no detectable sodium currents in a heterologous expression system. To further test the possible clinical consequences of reduced SCN3A activity, they investigated the effect of a hypomorphic Scn3a allele (Scn3a Hyp) on seizure susceptibility and behavior using a gene trap mouse line. Heterozygous SCN3A mutant mice ($SCN3A^{+/Hyp}$) neither exhibit spontaneous seizures nor hyperthermia-induced seizures, but they displayed increased susceptibility to electroconvulsive- and chemiconvulsive-induced seizures, which provide evidence that loss-of-function of SCN3A may contribute to increased seizure susceptibility [26].

2.4 Nav1.6

Nav1.6, mainly distributed to the soma and synaptic origin, is important for APs generation and propagation [27]. In the development process, Nav1.2 is gradually replaced by Nav1.6 in the mature node of Ranvier [28]. The first heterozygous missense mutation (p.Asn1768Asp) in the Nav1.6 gene SCN8A was identified in 2012 by whole-genome sequencing (WGS) in a patient with severe epileptic encephalopathy who exhibited early-onset seizures, autistic features, intellectual disability, ataxia, and sudden unexpected death in epilepsy (SUDEP) [29]. Since this initial discovery, more than 100 pathogenic SCN8A variants have been identified in patients with epilepsy [30]. Most of the SCN8A variants have been detected in individuals with EIEE.

Different mutations in the SCN8A gene encoding Nav1.6 have different effects on epilepsy. For the missense mutation V929F, an evolutionarily conserved residue in the pore loop of domain II of Nav1.6, it was found that heterozygous mutations produced well-defined spike-wave discharges and are associated to absence epilepsy in mice [31]. However, missense mutations in $Scn8a^{med-jo}$ were able to improve the epilepsy symptoms of $SCN1A^{+/-}$ heterozygotes. The mechanism might be the decrease in Nav1.6 expression of excitatory neurons compensating for the loss of Nav1.1 in inhibitory neurons [32]. Recently, more and more de novo and inherited SCN8A epilepsy mutations were detected by gene panel analysis [33]. For example, loss-of-function mutants [34], underlying the complex seizure phenotype, were identified using specific mouse line. It was suggested that decreasing Scn8a expression in cortical excitatory neurons could reduce seizures. On the contrary, the decreasing expression of SCN8A in the thalamic reticular nucleus (RT) leads to absence seizures. Loss of Scn8a will impair tonic firing mode behavior and impair desynchronizing recurrent RT-RT synaptic inhibition in the thalamic reticular nucleus, which suggested that Scn8a-mediated hypofunction in cortical circuits, conferring convulsive seizure resistance, while hypofunction in the thalamus is sufficient to generate absence seizures.

2.5 Nav1.7

The SCN9A gene encodes the Nav1.7 subtype, which was initially identified in the peripheral nervous system, sympathetic ganglion, and olfactory sensory neurons [35–38]. Nav1.7 is also found expressed in the central nervous system such as in the cerebral cortex and hippocampus [39]. A missense mutation of SCN9A (N641Y), at a conserved amino acid residue located at the intracellular loop between domain I and II, is associated with a family of febrile seizures (FS, N641Y). Mice carrying N641Y mutations were more susceptible to electrical stimulation-induced clonic and tonic seizures [40]. However, it is still unclear how SCN9A gene mutation caused epilepsy in the CNS.

3. Potassium channels

K^+ channels control the resting membrane potential and enable rapid repolarization of the AP by producing outward K^+ currents, thus limiting neuronal excitability. K^+ channels are composed of four pore forming a subunits and modulatory b subunits. Kv channels are the largest ion channel group (Kv1–Kv12) that are expressed substantially in the CNS. Dysfunction of Kv channels including Ca^{2+}-activated K^+ channels, are associated with epilepsy [2].

3.1 Large conductance calciumactivated potassium channel

Large conductance calcium-activated potassium (BK) channels, consisting of functional α subunit and the tissue-specific regulatory subunits (β1–4 and γ1–4), are widely distributed in the CNS. BK channels are usually considered as vital players in the development of epilepsy (**Figure 3**), with the evidence including the K^+ derangement and regulating AP shape and duration [41, 42].

Gain-of-function mutation of BK, promoting the high-frequency neuron firing, is associated with spontaneous epileptic seizures paradoxically in both humans and rodents [43]. In fact, patients suffering from generalized epilepsy were detected a site mutation D434G at the RCK1 domain of BK α subunit. D434G increased the opening time of BK, through the enhancement of Ca^{2+} sensitivity [43]. In terms of functionality, the enhanced membrane excitability is associated with the increased BK activity and fAHP consequent [43, 44]. The augment seems to be induced by an increased recovery rate, underlying fast currents of VGSCs with a APs' reduced refractory period and/or through disinhibiting thalamocortical circuits by blocking brain GABAergic interneurons [43, 45, 46].

The knockout mice of BK channel β4 subunit exhibit temporal lobe epilepsy (TLE) seizure associated with a gain-of-function phenotype of BK, which not only sharpens APs but also induces a higher neuronal firing frequency in hippocampus DG granule cells [47].It is worth mentioned that epileptic seizures themselves also could induce a gain-of-function effect to BK. Picrotoxin and pentylenetetrazol (PTZ) caused generalized tonic-clonic epileptic seizures, with giving rise to a gain-of-function effect on BK channels, presenting increased BK currents and neuron firing in the neocortex [48]. It is of interest that BK-specific inhibitors attenuated generalized tonic-clonic epileptic seizures in picrotoxin or PTZ-induced epilepsy models, which suppressed the increase of neuron firing [48, 49].

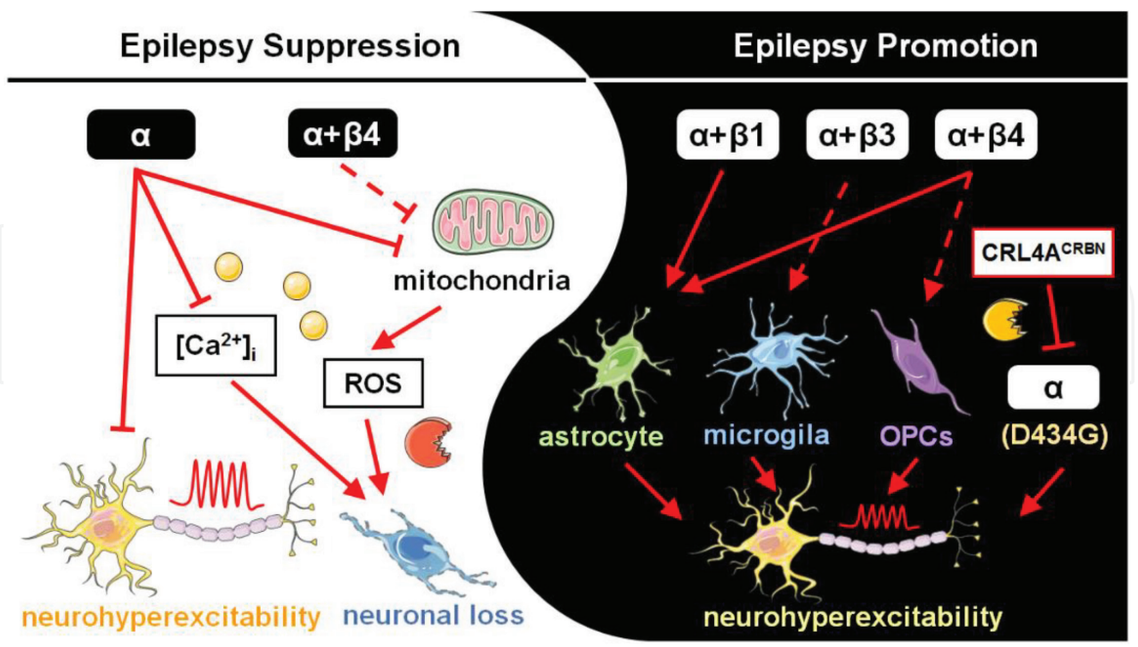

Figure 3.
Yin and Yang of BK channels in epilepsy. For epilepsy suppression, BK (α) channels act as negative feedback regulators on calcium rise and transmitter release in most synapses. Activation of mitoBK channel subtypes (α or α+β4) may contribute to suppressing seizure as well as conferring neuroprotection via the inhibition of ROS synthesis [54]. For epilepsy promotion, astrocyte and OPCs BK channel subtypes (α+β1 or α+β4) may induce elevate $[K^+]_o$, causing membrane depolarization as well as neuronal hyperexcitation. Microglial BK channels (α+β3) may involve in the neuroinflammation during status epilepsy. Mutation D434G of α causes the neurohyperexcitation in hereditary epilepsy. However, ubiquitin ligase $CRL4A^{CRBN}$ could inhibit the overactivation of BK channels.

Loss-of-function phenotype of BK might also contribute to the pathological process of clinical TLE. It was reported that two siblings suffered from the severe cerebellar atrophy and developmental delay, who adopted the exome analysis that identified a homozygous frameshift duplication in BK gene *KCNMA1* (c.2026dupT; p.(Tyr676 Leufs*7)) in children from a consanguineous family with epilepsy [50].

KCNMB3, encoding the auxiliary BK β3, mapping the human chromosome 3 (3q26.3-q27) [51], is duplicated in the dup (3q) syndrome, which is characterized by neurological abnormalities, especially epileptic seizures [51]. Because of the dup (3q) syndrome having early onset during developmental process, the *KCNMB3* duplication implies that β3 subunits overexpression might contribute to the etiology of epilepsy. Similarly, site mutations might also contribute to both neurohyperexcitation by a single nucleotide deletion at *KCNMB3* exon 4 (delA750), which is associated with the generalized epilepsy, especially in the form of the typical absence epilepsy [52]. BK coexpressed with β3 variant of β3b-V4 (delA750) shows fast inactivation properties [53], which suggest that BK currents were reduced and the repolarization of cell membrane was attenuated during an action potential, eventually leading to neurohyperexcitation.

3.2 Voltage-gated potassium channel subfamily KQT (KCNQ)

Kv7 is its seventh member of Kv channel family (Kv1–Kv12). The Kv7.1 mutation mediates type 1 long QT syndrome (long-QT syndrome type 1, LQT1) and is therefore named KCNQ1 (K, potassium; CN, channel; Q, LQT). KCNQ has five subtypes of KCNQ1–KCNQ5, which play crucial roles in physiological functions. Dysfunction of KCNQ is associated with many diseases.

KCNQ1 is mainly distributed in the heart, which mediates cardiac delayed-rectifier K^+ current and maintains the normal repolarization process of cardiomyocytes [55]. KCNQ2–KCNQ5 are mainly distributed in central and peripheral neuronal tissues, of which KCNQ2 and KCNQ3 are distributed in brain regions [56]. KCNQ2 and KCNQ3 form functional heterotetramers, which are the main molecular bases for the formation of M currents that can be inhibited by acetylcholine M1 receptor activation [57]. Abundant KCNQ2 and KCNQ3 mutations could induce abnormal M currents, causing similarities in neonatal seizures and other nervous system diseases.

Benign familial neonatal seizure (BFNS) is an autosomal dominant idiopathic epilepsy syndrome that occurs on the 2nd to 8th day after birth and stops spontaneously after a few weeks. Whereas 15% of patients in later life may have recurrence of epilepsy [58]. With the study of pathogenic genes in epilepsy, 60–70% of patients with BFNS were found to be associated with KCNQ2 and KCNQ3 mutations. More than 80 different mutations have been reported on KCNQ2, and multiple mutations on KCNQ3 are associated with BFNS. Soldovieri et al. [58] studied the genes of 17 BFNS clinical patients. Sixteen different heterozygous mutations were found in KCNQ2, including 10 substitutions, 3 insertions/deletions, and 3 large deletions. One substitution was found in KCNQ3. Most of these mutations were novel, except for four KCNQ2 substitutions that were shown to be recurrent. Electrophysiological studies in mammalian cells revealed that homomeric or heteromeric KCNQ2 and/or KCNQ3 channels carrying mutant subunits with newly found substitutions displayed reduced current densities. Borgatti studied a BFNS family with four affected members: two of them exhibit BFNS only, while the other two, in addition to BFNS, present either with a severe epileptic encephalopathy or with focal seizures and mental retardation. All affected members of this family carry a novel missense mutation in the KCNQ2 gene (K526N), disrupting the tridimensional conformation of a C-terminal region of the channel subunit involved in accessory protein binding. When heterologously expressed in CHO cells, potassium channels containing mutant subunits in homomeric or heteromeric configuration with wild-type KCNQ2 and KCNQ3 subunits

exhibit an altered voltage-dependence of activation, without changes in intracellular trafficking and plasma membrane expression. The KCNQ2 K526N mutation might affect M-channel function by disrupting the complex biochemical signaling involving KCNQ2 C-terminus [59, 60]. KCNQ2 or KCNQ3 mutations cause M current to be downregulated, and the frequency of neuronal firing increases, leading to epilepsy.

3.3 G protein-coupled Kir channel

Inward-rectifier potassium channels (Kir, IRK) are a specific subset of potassium channels. To date, seven subfamilies have been identified, which are associated with a variety of diseases [61]. The G-protein-coupled Kir (GIRK) channels belong to the subfamily of Kir3 (GIRKs) which are activated by ligand-stimulated G protein-coupled receptors (GPCRs). GPCRs, interacting with GIRK channels, facilitate their activation, resulting in hyperpolarization of the cell membrane [61].

GIRK channels have four identified subunits (GIRK1–4, encoded by KCNJ3, KCNJ6, KCNJ9, and KCNJ5, respectively) in mammals, existing in vivo both as homotetramers and heterotetramers with unique biophysical properties, regulation, and distribution [61, 62]. GIRK 1, 2, 3, and 4 subunits are expressed in the brain, localized in certain axons, postsynaptic, and presynaptic regions [63]. GIRK channels may be involved not only in slow inhibitory postsynaptic potentials but also in the presynaptic modulation of neuronal activity [61].

GIRK in the CNS is a heterotetramer composed of GIRK1 and GIRK2 subunits [63], which is responsible for maintaining the resting membrane potential and excitability of the neuron [64]. GIRK1 and GIRK2 subunits are found in the dendritic areas of neurons highly [63] correlate with the large concentration of $GABA_B$ receptors. Once the $GABA_B$ receptors are activated by their ligands, they can in turn activate IRK, mediating a significant part of the GABA postsynaptic inhibition [63].

Alterations in GIRK function have been associated with pathophysiology of severe brain disorders, including epilepsy. In this regard, a GIRK2 knockout mouse model resulted to be more susceptible to develop both spontaneous and induced seizures in respect to wild-type mice [65]. In particular, mice carrying a p Gly156Ser mutation displayed an epileptic phenotype [66]. Indeed, this mutation has been found to alter the putative ion-permeable, pore-forming domain of the channel, inducing Ca^{2+} overload in cells and reducing channel availability, leading thus to neurodegeneration and seizure susceptibility [67].

An increased expression of GIRK was observed in rat brain after an electroconvulsive shock, probably altering the excitability of granule cells and the functions of neurotransmitter receptors which are coupled to these channels [68]. Another evidence in support of a role of GIRK in epilepsy was provided by the demonstration that ML297, a potent and selective activator of GIRK, showed epileptogenic properties in mice [69]. On the other hand, the inhibition of GIRK activity by drugs causes seizures [70]. All these considerations imply that changes in Kir3 channel activity may alter the susceptibility to seizures.

4. Calcium channels

As an important second messenger, Ca^{2+} plays a vital role in normal brain function and in the pathophysiological process of different neurodegenerative diseases. Ca^{2+} entry via VGCCs conveys the electric signals to intracellular transduction cascades in a wide variety of cells [71]. VGCCs were first identified by Fatt and Katz [72] and shown to consist of several subunits [73, 74]. VGCCs were divided into low-voltage-activated (LVA) and high-voltage-activated (HVA), based on electrophysiological and

pharmacological properties. HVA channels, composed of α_1, β, $\alpha_2\delta$, γ subunit, are further divided into L, N, P, and Q types, which have an activation threshold at membrane voltage positive to −20 mV [75]. LVA channels, also called T type, consist only of the α_1 subunit, activated at a membrane voltage positive to −70 mV. It is composed of transmembrane topology with four homologous transmembrane domains, each containing six transmembrane segments and a pore region between segments S5 and S6.

4.1 L-type Cav

The L-type VGCC family has four members, Cav1.1–1.4, of which α subunits present tissue-specific expression, such as the α_1D subunit in the brain. The L-type VGCC family shapes neuronal firing and activates Ca^{2+}-dependent pathways involved in regulation of gene expression [76]. Cav1.2 channels appear to contribute critically to the generation of febrile seizures, which was proved by testing the excitability of hippocampal pyramidal cells in rat brain slices [77]. The Wistar Albino Glaxo/Rij (WAG/Rij) model experiments suggest that L-type calcium channels play a positive role in the frequency and duration of epileptic spikes [78]. Verapamil, an L-type VGCC blocker, could significantly reduce TLE seizure, enhancing the expression of the α subunit of γ-$GABA_AR$ [79].

4.2 P/Q-, N-, and R-type Cav

P/Q-, N-, and R-type are corresponding to Cav2.1, Cav2.2, and Cav2.3, respectively, which initiate rapid synaptic transmission, regulated primarily by direct interaction with G proteins and SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) and secondarily by protein phosphorylation. The loss function of P/Q VGCC could lead to epileptic spikes, paroxysmal dystonia and ataxia. If P/Q VGCCs were blocked, it could disrupt the triggering synaptic neurotransmitter release [80]. Spikes of Cacna1aNtsr$^{-/-}$ mice are increased in layer VI corticothalamic neurons compared with control group, suggesting that Cav P/Q deletion generates absence epilepsy [81]. Cacna1a LOF from parvalbumin (PV)(+)
and somatostatin (SST)(+) interneurons results in severe generalized epilepsy. It might be the mechanism for severe generalized epilepsy that the loss of Cav2.1 channel function from cortical PV(+) interneurons inhibits GABA release from these cells, which impairs their ability to constrain cortical pyramidal cell excitability [82]. When knocking out the cerebellar Cav2.1 channel in mice, cortical function is changeable, which caused movement disorders and epilepsy [83]. In two families with idiopathic epilepsy, the loss of function mutation in γ4 subunits, auxiliary subunit of Cav2.1 channels, could also cause seizures, and maybe aggravate seizures [84]. Downregulation of α2δ2 subunits in rats will generate 5–7 Hz epileptic wave accompanied by ataxia [85]. N-type calcium channels are mainly distributed in the nucleus of different neurons and glial cells. In the pilocarpine model, Cav2.2 expression decreased in the granule layer of the dentate gyrus and the pyramidal cells of the CA3 region during the acute phase of seizure. However, the expression of N-type calcium channels increased in the subsequent chronic phase, which demonstrated that the increase of N-type calcium channels might be associated with recurrent status epilepticus [86]. R-type calcium channel, Cav2.3, is mainly distributed in the presynaptic membrane, such as hippocampal mossy fibers, globus pallidus, and neuromuscular junctions. Knocking out R-type calcium channels could increase the susceptibility of seizures, with altering the seizure form [87]. The lack of Cav2.3 resulted in a marked decrease in the sensitivity of the animal to γ-butyrolactone-induced absence epilepsy and change thalamocortical network oscillations [88]. Administration of kainic acid revealed alteration in behavioral seizure architecture, dramatic resistance to limbic seizures

and excitotoxic effects in Cav2.3$^{-/-}$ mice compared with controls. It indicated that the Cav2.3 plays a crucial role in both hippocampal ictogenesis and seizure generalization and is of central importance in neuronal degeneration after excitotoxic events [89].

4.3 T-type Cav

T-type channels, widely distributed in the thalamus, are important for the repetitive firing of APs in rhythmically firing cells, which could be activated and inactivated more rapidly at more negative membrane potentials than other VGCCs [90]. Three subtypes of T-type channels have been identified, designated as Cav3.1, Cav3.2, and Cav3.3; they correspond to complexes containing the pore-forming α1 subunits, α1G, α1H, and α1I, respectively [91]. It has long been suggested that generalized absence seizures are accompanied by hyperexcitable oscillatory activities in the thalamocortical network [92]. The evidence that succinimide and related anticonvulsants could block thalamic T-type channels make researchers speculate that T-type Ca2+ channels might be related to the pathogenesis of spike-and-wave discharges (SWDs) in generalized absence seizures [93]. In the kainate epilepsy model, Cav3.1$^{-/-}$ mice display significantly reduced duration of seizures compared to wild type, but the frequency of seizures increased slightly [94]. In the WAG/Rij model, the expression of Cav3.1 may be related to age, and blocking Cav3.1 can reduce the onset of epilepsy [94, 95] which suggested that decrease in Cav3.1 channel expression and Ca^{2+} current component that they carry in thalamocortical relay neurons serves as a protective measure against early onset of SWD and absence seizures [96]. Notably, Cav3.1$^{-/-}$ mice are resistant to SWD seizures specifically induced by γ-$GABA_B$R agonists. Simultaneously, the γ-$GABA_B$R agonists induced only very weak and intermittent SWDs in Cav3.1$^{-/-}$ mice [97]. Cav3.2 single nucleotide mutation has been reported in patients with childhood absence epilepsy and other types of idiopathic generalized epilepsies [98, 99]. Gain-of-function mutations (C456S) in Cav3.2 channels increase seizure susceptibility by directly altering neuronal electrical properties and indirectly by changing gene expression [100].

5. Transient receptor potential channels

Transient receptor potential (TRP) channels, which could induce a transient voltage changes to continuous light mutations of *Drosophila melanogaster*, are expressed in photoreceptors carrying trp gene. The first homologous human gene was reported in 1995. There are 30 trp genes, and more than 100 TRP channels have been identified so far, and TRP channels were divided into 7 subfamilies, including TRPC, TRPV, TRPM, TRPA, TRPP, TRPML, and TRPN. Focus on TRPs, one family of Ca^{2+} channels, plays a role in neuronal excitability. It is obviously known that Ca^{2+} is an important second messenger, which is related to the etiology of epilepsy [101]. Therefore, TRP channels are thought to be partially responsible for epileptic seizures, especially for TPRC and TRPV1 channels.

5.1 Canonical transient receptor potential (TRPC)

TRPC channels are the closet homolog to Drosophila TRP channels. Based on the functional comparisons and sequence alignments, four subsets of mammalian TRPCs (TRPC1, TRPC2, TRPC3/6/7, and TRPC4/5) have been generated [101]. These channels form receptor-modulated currents in the mammalian brain and important to SE-induced neuronal cell death. These channels could play a critical role in the generation of spontaneous seizures. TRPC1 and TRPC4 are expressed in CA1 pyramidal neurons. The amplitude of the plateau and the number of spikes

were significantly reduced in mice without TRPC1 and TRPC4 [102]. TRPC3 channels are found to be responsible for pilocarpine-induced status epilepticus (SE) in mice. The reduction on SE in TRPC3 KO mice is caused by a selective attenuation of pilocarpine-induced theta wave activity [103]. TRPC7 can be detected in CA3 pyramidal neurons largely. The spontaneous seizures in CA3 pyramidal neurons and the pilocarpine-induced increase in gamma wave activities during the latent period could be significantly reduced by ablating the gene TRPC7 [104].

5.2 Transient receptor potential vanilloid 1 (TRPV1)

TRPV1 is one subfamily of TRP channels, expressing in most neurons. The expression of TRPV1 protein in epileptic brain areas was increased [105], but the epileptic activity in hippocampal slices was decreased by iodoresiniferatoxin (IRTX), a selective TRPV1 channel antagonist [106]. It is well known that glutamate could be released when the TRPV1 channel was activated [107], and the glutamate neurotransmitters are related to the etiology of epilepsy. Thus, focusing the TRPV1 channels activity may be important for the modulating neuronal excitability in epilepsy [106]. Recent studies showed that the high expression of TRPV1 channels could induce the temporal lobe epilepsy [105]. Cytosolic calcium elevation through activation of TRPV1 channels plays a physiologically relevant role in the regulation of epileptic seizures [108], decreasing the calcium accumulation by inhibiting the TRPV1 channels, could play a neuronal protective role against epilepsy-induced Ca^{2+} entry in hippocampal neurons. As mentioned above, the TRPV1 could be activated by hyperthermia; the hyperthermia-induced TRPV1 might be an effective candidate therapeutic target in heat-induced hyperexcitation [109, 110]. The activation of TRPV1 promotes glutamate release by increasing the excitability of neurons and synaptic terminals [111]. Whereas the activities would be reduced in hippocampus slices of rats after given the CPZ and ITRX, which were the TRPV1 channel blockers.

6. Antiepileptic therapy and beyond

At present, the treatment of epilepsy is still dominated by drugs. More than 35% of marketed antiepileptic drugs target VGICs, such as phenytoin, carbamazepine, oxcarbazepine, and ethosuximide. Phenytoin and carbamazepine are broad-spectrum antiepileptic drugs blocking VGSCs as their primary mechanism of action. For example, phenytoin is a more effective inhibitor of SCN8A-I1327V than other drugs [112], which could be used in treating patients with gain-of-function mutations of SCN8A. Different types of VGCCs play different roles in the pathological process of epilepsy. Decreased expression of P/Q type could induce epilepsy, whereas increased expression of N-type and T-type calcium channels could lead to epilepsy. Calcium blockers including ethosuximide have been widely accepted for the treatment of absence epilepsy [71]. Gain-of-function BK channels contribute to epileptogenesis and seizure generation. BK-blocking agents, like paxilline [49], might be used as potential therapeutic drugs.

In the future, novel techniques might contribute to develop reasonable therapies for treating inherited or acquired epileptic syndromes. For instance, induced pluripotent stem cells (IPS) and genetically engineering animal models could be used for accurate treatments of epilepsy. Single-nucleotide polymorphisms (SNPs) of VGIC genes from hereditary epilepsy patients could be detected by *de novo* genomic sequencing. VGICs of IPS cells could be mutated by CRISPR-Cas9 according to the information of these SNPs [113]. Through inducing IPS cells differentiated into neurons, phenotype of VGIC gene SNPs could be well investigated. It is also a well-detection platform for selecting antiepileptic drugs that would be sensitive to mutated VGICs *in vitro* [112]. For *in vivo*

tests, besides transgenic mice, construction of nematode or zebrafish epileptic models may be creating a shortcut for choosing suitable and personalized antiepileptic drugs [114, 115]. In addition to drug control, optogenetics and ultrasonic control are hopeful to suppress the epileptic seizures induced by VGIC dysfunction [116, 117].

7. Conclusion

We systemically summarized the mutations and phenotype information of 21 epilepsy-associated VGIC genes. The dysfunctional VGICs are like the blasting fuse for neuronal hyperexcitability. We have good reason to believe that epilepsy-associated mutations of VGICs could be considered as a biomarker, which is possible to be one of the molecular bases underlying the classification of epilepsy syndromes identified by modern medicine. VGICs are the important targets for many antiepileptic drugs. Novel VGIC modulators are potentially effective strategy for the development of novel antiepileptic drugs. Individualized precise treatment using matching VGIC drugs will provide novel research directions and antiepileptic strategies.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

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