Neurosci. Bull. August, 2017, 33(4):455–477
DOI 10.1007/s12264-017-0134-1

www.neurosci.cn
www.springer.com/12264

REVIEW

Ion Channel Genes and Epilepsy: Functional Alteration, Pathogenic Potential, and Mechanism of Epilepsy

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Received: 29 December 2016 / Accepted: 20 February 2017 / Published online: 9 May 2017
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Abstract Ion channels are crucial in the generation and modulation of excitability in the nervous system and have been implicated in human epilepsy. Forty-one epilepsy-associated ion channel genes and their mutations are systematically reviewed. In this paper, we analyzed the genotypes, functional alterations (funotypes), and phenotypes of these mutations. Eleven genes featured loss-of-function mutations and six had gain-of-function mutations. Nine genes displayed diversified funotypes, among which a distinct funotype-phenotype correlation was found in *SCN1A*. These data suggest that the funotype is an essential consideration in evaluating the pathogenicity of mutations and a distinct funotype or funotype-phenotype correlation helps to define the pathogenic potential of a gene.

Keywords Epilepsy · Ion channel gene · Epilepsy gene · Genetics · Gene function · Pathogenic mechanism

Introduction

Epilepsy is a group of chronic brain disorders characterized by recurrent seizures due to abnormal excessive electrical discharges of cerebral neurons [1]. It is generally believed that genetic factors play an important role in the etiopathogenesis of epilepsy. Recent studies have demonstrated that 977 genes are associated with epilepsy, among which genes encoding ion channels predominate [2].

Ion channels are pore-forming membrane proteins. Their functions include establishing action potentials and maintaining homeostasis by gating the ionic flow traversing the cell membrane, managing the ionic flow across cells, and regulating cell volume. Since these functions are essential to the excitability of neuron, ion channels potentially play a critical role in epileptogenesis [3]. The association between ion channel genes and epilepsy may provide insights into the mechanisms underlying epilepsy.

CHRNA4, which encodes the α4 subunit of the ligand-gated ion channel nAChR (nicotinic acetylcholine receptor), was the first epilepsy gene identified in patients with autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE) in 1995 [4]. Since then, many ion channel genes have been reported to be potentially associated with epilepsy [2]. However, the associations differ. Genes like *CHRNA4* have been confirmed to be epilepsy genes by familial co-segregation, multi-source validations, and functional alteration [4–7], whereas some genes warrant further investigation. Functional studies are used to determine the impairments caused by gene mutations and provide insights into the underlying mechanism of epilepsy. Evidence from functional studies is also helpful in evaluating the pathogenicity of a gene and its mutations, especially when considered together with clinical features.

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In this review, we summarize the epilepsy-associated ion channel genes, the mutations, the functional changes in mutants, and the corresponding phenotypes and inheritance, aiming to provide clues for evaluating the association between ion channel genes and epilepsy and understanding the mechanisms of epilepsy.

Gene Searching and Analysis Strategies

Based on several databases (OMIM (Online Mendelian Inheritance in Man), HGMD (Human Gene Mutation Database), and EpilepsyGene) and recent publications in PubMed, we previously retrieved 977 epilepsy-associated genes [2], among which 60 are ion channel genes, including 28 epilepsy genes and 32 potential epilepsy genes (not yet in the OMIM database). We systematically searched all the publications for mutational and functional studies of these genes. For each gene, we searched the PubMed database using the terms gene symbol, gene full name, and the corresponding gene-encoded product, like "*CHRNA4*", "cholinergic receptor nicotinic alpha 4 subunit", and "nAChRα4" or "α4 subunit of nAChR". Additional searches were performed according to the reference list of the publications. We included all epilepsy genes and potential epilepsy-associated genes with functional studies performed on their mutants. For genes with updated reviews, such as *SCN1A*, we summarized the data from recent publications [8].

Functional alterations (functional types, or funotypes) are generally classified into gain-of-function (GOF), loss-of-function (LOF; that refers to the complete loss of function), and partial loss-of-function (pLOF; that denotes mutants with residual current (function)) as in our previous report [9]. To facilitate understanding of the functional consequences, gene mutations are classified into destructive and missense mutations. Destructive mutations are those causing gross protein malformations, including truncating mutations (nonsense and frameshifting mutations), splice-site mutations, and mutations with genomic rearrangement, which mainly lead to functional deficiency and haploinsufficiency. The functional consequences of missense mutations need to be determined in biophysiological studies.

When referring to animal models of these genes, data from mutation-specific and phenotype-related models are described and cited in this work. More information on genetic knock-out or knock-in mouse models can be retrieved from the Mouse Genome Informatics database (http://www.informatics.jax.org/).

Gene Mutations, Functional Alterations, and Pathogenic Mechanisms

Forty-one ion channel genes were included in the analysis, covering 28 epilepsy genes, one epilepsy-related gene (*GRIN1*), and 12 potential epilepsy genes. Based on their biophysical and physiological characteristics, these genes were classified into eight main groups (Table 1). More than 1,600 mutations have been identified in these genes, most of which are associated with more than one epileptic phenotype. In the following we analyze the corresponding mutations and their functional changes in each phenotype.

Sodium Channel Genes

Voltage-gated Na^+ channels in the brain are composed of one large pore-forming α subunit and two smaller β subunits [10]. They are critical for neuronal excitability, including action potential initiation and conduction [11]. The α-subunit is capable of conducting currents on its own, and is expressed in a tissue-specific manner. *SCN1A* (encoding $Na_V1.1$), *SCN2A* (encoding $Na_V1.2$), *SCN3A* (encoding $Na_V1.3$), *SCN8A* (encoding $Na_V1.6$), and *SCN9A* (encoding $Na_V1.7$) have been associated with epilepsy. The β-subunits modulate multiple aspects of Na_V channel behavior and are essential for the control of neuronal excitability [12]. *SCN1B* (encoding $Na_V\beta1$) is an epilepsy gene (Table 2).

SCN1A is expressed at a high level in the central nervous system (CNS), and $Na_V1.1$ is found predominantly in the somata and dendrites of neurons [13]. *SCN1A* is one of the most important causative genes in epilepsy. To date, >1,257 epilepsy-related mutations have been reported [8], mainly in patients having epilepsy with antecedent febrile seizures (FS). Severe myoclonic epilepsy (SME) in infancy is the most severe phenotype and is frequently associated with destructive or missense mutations located in the pore region which cause LOF of $Na_V1.1$. In contrast, mild generalized epilepsy with febrile seizures plus (GEFS+) or FS has the highest frequency of missense mutations that are usually located outside the pore region and cause mild functional changes. Partial epilepsy with FS+ (PEFS+) is an intermediate phenotype in terms of both clinical severity and mutation impairment. These data suggest that LOF of $Na_V1.1$ is the primary basis for epilepsies with FS+, and the clinical severity is correlated with the functional impairment in a quantity-dependent manner. Experiments in *Scn1a* knock-out mice have demonstrated that the Na^+ current density is reduced in inhibitory interneurons, but not in excitatory pyramidal neurons, explaining how the LOF of $Na_v1.1$ would impair inhibitory functions in the brain and lead to hyperexcitability and epilepsy. Inhibitory interneurons are generally distributed locally with heterogeneity in different brain areas [14], explaining the common partial seizures in SME and PEFS+ [8, 15–17].

SCN1B encodes $Na_V\beta1$ that can influence many cardinal conformational changes of Na_V channels during the action potential process [18]. *SCN1B* mutations were initially

Table 1 Summary of human ion channels implicated in epilepsies.

Gating categories	Main functions	Gene (Protein)
Sodium Channels	Responsible for generation and propagation of action potentials	*SCN1A* ($Na_V1.1$), *SCN1B* ($Na_V\beta1$), *SCN2A* ($Na_V1.2$), *SCN3A* ($Na_V1.3$), *SCN8A* ($Na_V1.6$), *SCN9A*†($Na_V1.7$)
Potassium Channels		
Voltage-gated	Regulation of outward K^+ currents and action potentials, modulation of neurotransmitter release	*KCNA2* ($K_V1.2$), *KCNB1* ($K_V2.1$), *KCNC1* ($K_V3.1$), *KCND2* ($K_V4.2$), *KCND3* ($K_V4.3$), *KCNH2* ($K_V11.1$), *KCNH5* ($K_V10.2$), *KCNQ2* ($K_V7.2$), *KCNQ3* ($K_V7.3$), *KCNV2* ($K_V8.2$)
(Ca^{2+}-activated)	Regulation of neuronal firing properties and circuit excitability	*KCNMA1* ($K_{Ca}1.1$)
(Na^+-activated)	Regulation of delayed outward I_{KNa} currents and contribution to adaptation of firing rate	*KCNT1* ($K_{Ca}4.1$)
Calcium channels	React to membrane potential depolarization by opening and provide an elevation of Ca^{2+} ions to drive many processes	*CACNA1A* ($Ca_V2.1$), *CACNA1H* ($Ca_V3.2$), *CACNA2D2*#($Ca_V\alpha2\delta$-2), *CACNB4* ($Ca_V\beta4$),
Chloride channels	Maintenance of resting membrane potential and regulation of cell volume	*CLCN2* (CLC-2), *CLCN4* (CLC-4)
γ-Aminobutyric acid type A receptor	Mediation of major inhibitory functions in CNS	*GABRA1* ($GABA_A\alpha1$), *GABRA6* ($GABA_A\alpha6$), *GABRB1* ($GABA_A\beta1$), *GABRB2* ($GABA_A\beta2$), *GABRB3* ($GABA_A\beta3$), *GABRD* ($GABA_A\delta$), *GABRG2* ($GABA_A\gamma2$)
Ionotropic glutamate receptors	Excitatory synaptic transmission, plasticity, and excitotoxicity of the CNS	*GRIN1* (GluN1), *GRIN2A* (GluN2A), *GRIN2B* (GluN2B), *GRIN2D* (GluN2D)
Nicotinic acetylcholine receptors	Permeation of Na^+ and K^+ and modulation of neurotransmitter release	*CHRNA2* (nAChRα2), *CHRNA4* (nAChRα4), *CHRNA7* (nAChRα7), *CHRNB2* (nAChRβ2)
Hyperpolarization-activated cyclic nucleotide-gated channels	Permeation of Na^+ and K^+	*HCN1* (HCN1), *HCN2* (HCN2)

Underlined: potential epilepsy-associated genes with functional alterations examined.

† *SCN9A* may be one of the digenic causes of Dravet Syndrome with *SCN1A*.

CACNA2D2 may be one of the digenic causes of epilepsy with *CELSR3*.

identified in families with epilepsy and FS. Functional studies on mutants (R85C, R85H, C121W, and R125C) revealed LOF of β1 and subsequently impaired function of Na^+ channels [11, 19, 20]. Two homozygous missense mutations (I106F and R125C) have been identified in more severe cases (SME patients) [21, 22], indicating a quantity-dependent feature. The *SCN1B* phenotype shows clinical features similar to those of *SCN1A*, suggesting that the mechanism underlying the pathogenicity of *SCN1B* mutations potentially involves impaired function of $Na_V1.1$.

The temporal expression pattern of *SCN2A* in the brain is similar to *SCN1A*, but $Na_V1.2$ is specifically localized in axons and terminals [13]. *SCN2A* mutations were initially identified in families with benign familial neonatal-infantile seizures [23, 24]. Functional studies showed pLOF with decreased channel availability in two mutants and GOF in another two mutants [23–28]. It is hard to explain the pathogenicity of heterozygous mutations with pure LOF or pLOF, since heterozygous knock-out of *Scn2a* in mice does not result in seizure activity [29]. There is no mutation-specific knock-in model to show whether a mutation with GOF would be pathogenic. *SCN2A* is transcribed in different splice forms during neonatal and adult stages. The neonatal splice isoform is less excitable than that of adults, and mutants would change the channels to a more excitable status than the neonatal isoform but at a level similar to adult channels [30]. This may be one of the explanations for the pathogenicity of *SCN2A* mutations in neonates. Multiple *de novo* mutations have been identified in patients with epileptic encephalopathies (EEs) through next-generation sequencing. However, their roles in the pathogenicity of EEs are currently uncertain due to a lack of evidence.

Scn3a in rodents is expressed at the highest level in the embryonic and early postnatal brains and gradually disappears thereafter [31]. In contrast, *SCN3A* is expressed in small amounts in the adult human brain, and $Na_V1.3$ shows a somatodendritic localization [32]. *SCN3A* has been potentially associated with epilepsy in several publications [33–35]. Functional analyses have shown GOF in three [33, 34], pLOF in one [35], and no significant changes in two of the mutants [34]. The functional changes are generally slight in these mutants. The relationship between *SCN3A* and epilepsy remains to be clarified.

Table 2 Mutations in epilepsy-associated Na^+ channel genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
SCN1A	SME	*de novo* (mostly), inherited	42.3% cases have missense mutations 57.7% cases have destructive mutations	Missense mutations in pore region (54.1%) lead to LOF or pLOF, in other regions can cause pLOF, G-LOF, and LOF	[8]
	PE and/or FS+	*de novo*, inherited	74.5% cases have missense mutations 25.5% cases have destructive mutations	Missense mutations in pore region (42.1%) lead to LOF or pLOF, in other regions can cause pLOF, G-LOF, and LOF	
	GE and/or FS+	Inherited (mostly), *de novo*	87.0% cases have missense mutations 13.0% cases have destructive mutations	Milder functional alterations, such as increased excitability, decreased excitability, pLOF, or pure GOF, but no LOF and G-LOF	
SCN1B	EFS+	AD	R85C, R85H, C121W	pLOF	[11, 19, 20]
			R125L, K208I	Not available	
			c.208-2A>C	Destructive	
	IE	Unknown	T28I	Not available	
	PS	Unknown	D25N	Not available	
	SME	AR	R125C (homozygous)	LOF	[22]
			I106F (homozygous)	Not available	
SCN2A	BFNS	AD	R1319Q, L1330F	pLOF	[25]
			L1563V, M252V	GOF	[25, 26]
			>12 missense mutations	Not available	
			1 gross insertion and 2 gross deletions	Destructive	
		de novo	V261M	GOF	[26]
	FS and GEFS+	AD	R188W	pLOF	[188]
	EE	*de novo*	A263V	GOF	[189]
			R102X	Destructive, LOF	[27]
			>25 missense mutations	Not available	
			2 gross insertions	Destructive	
SCN3A	CPS	Paternal†	K354Q	GOF	[33]
		Unknown	E1111K	GOF	[34]
			R357Q, D766N	Unchanged	[34]
	PEFS+	Unknown	M1323V	GOF	[34]
	GEFS+	*de novo*	N302S	pLOF	[35]
SCN8A	EE	*de novo*	T767I, N984K, N1768D, R1617Q, R1872W, R1872L, R1872Q	GOF	[37, 40]
			R223G	pLOF, thermolabile mutant	[39]
			G1451S	LOF at 37°C	[40]
			>30 missense mutations	Not available	
			2 deletions	Destructive	
SCN9A	SME	Maternal†	I228M#	GOF	[44]
			I684M#, L1123F#, E519K, E1160Q	Not available	
		Paternal†	K655R#, I739V#, C699Y	Not available	
		Unknown	I1267V#, K655R	Not available	
	FS and FS+	AD	N641Y	GOF (in mouse model)	[45]
		Paternal†	I739V	Not available	
		Unknown	I62V, P149Q, K655R, S490N	Not available	

AD, autosomal dominant; AR, autosomal recessive; BFNS, benign familial neonatal seizures; CPS, complex partial seizure; EE, epileptic encephalopathy; FS, febrile seizure; GE, generalized epilepsy; GEFS+, generalized epilepsy with febrile seizures plus; GOF, gain-of-function; IE, idiopathic epilepsy; LOF, loss-of-function; pLOF, partial loss-of-function; PE, partial epilepsy; PEFS+, partial epilepsy with febrile seizures plus; PS, partial seizure; SME, severe myoclonic epilepsy.

† Incomplete penetrance; transmitter not affected.

Combined with *SCN1A* mutation.

SCN8A is highly expressed in cerebellar granule cells and in pyramidal and granule cells of the hippocampus [13]. *SCN8A* has been associated with EEs in recent years. More than 40 *de novo SCN8A* mutations have been identified in cases with various EEs. All the mutations are missense, except two that are destructive. Nine of the missense mutations have been characterized in functional studies. A majority of the mutations, including T767I, N984K, N1768D, R1617Q, R1872W, R1872L, and R1872Q, have demonstrated GOF; whereas G1451S and R223G display LOF or pLOF with thermosensitivity [36–40]. No distinct genotype-phenotype or funotype-genotype association has been found. Considering that *Scn8a*-null heterozygote mice are seizure-resistant [41, 42], mutants with LOF are unlikely to be pathogenic. Further studies are required to determine the role of *SCN8A* mutations in EEs and the underlying mechanism.

SCN9A is expressed predominantly in the peripheral nervous system and slightly in the CNS. The first suspicion of an association between *SCN9A* and epilepsy came from linkage analysis that located an FS-related locus in the genomic region containing *SCN1A*, *SCN2A*, and *SCN3A*[43], and *SCN9A* is located nearby. Several *SCN9A* mutations have been identified in patients with FS-related epilepsies [44, 45]. In an SME cohort, six of nine patients with *SCN9A* missense variants also harbored *SCN1A* mutations [45], suggesting that *SCN9A* may be one of the digenic causes of SME. A mouse model with knock-in of N641Y presents susceptibility to epileptic seizures, suggesting that *SCN9A* may be a modifier or susceptibility gene of epilepsy [45].

Although voltage-gated Na^+ channels have molecular and physiological characteristics in common, their associations with epilepsy differ in many aspects, including phenotype, pathogenic funotype, and the underlying pathogenic mechanism.

Potassium Channel Genes

K^+ channels control the resting membrane potential and enable rapid repolarization of the action potential by producing outward K^+ currents, thus limiting neuronal excitability [46]. K^+ channels are composed of four pore-forming α subunits and modulatory β subunits. Voltage-gated K^+ (K_V) channels are the largest ion channel group that are expressed substantially in the CNS. K_V channels, including Ca^{2+}-activated and Na^+-activated K^+ channels, have been associated with epilepsies (Table 3).

KCNA2 encodes $K_V1.2$ that is expressed in axons and synaptic terminals; it enables efficient repolarization following an action potential [47]. Five missense mutations within *KCNA2* have been identified in patients with EEs [47, 48]. Functional studies of the mutations I263T and P405L have shown LOF with a dominant-negative effect [47], predicting hyperexcitable neuronal membranes and repetitive firing due to impaired repolarization. *Kcna2*-knock-out mice display increased seizure susceptibility and premature death, supporting the role of LOF mutants in epilepsy [49]. Another two mutations (R297Q and L298F) demonstrate GOF, predicting permanently open channels at physiological membrane potentials and electrical silencing by membrane hyperpolarization [47]. Further studies are required to elucidate the mechanism of action of $K_V1.2$ GOF mutants in epileptogenesis.

KCNB1 encodes $K_V2.1$, which is the main contributor to the delayed rectifier K^+ current in pyramidal neurons of the hippocampus and cortex [50]. This current is vital for membrane repolarization and for suppressing high-frequency firing. Nine *KCNB1* mutations have been reported in EE patients, and most of the mutations are located in the pore region [51–53]. Six mutations show LOF, and four of them (S347R, T374I, V378A, and G379R) also cause a loss of K^+ selectivity with a dominant-negative effect [51]. Considering the function of suppressing high-frequency firing, LOF of $K_V2.1$ predicts hyperactivity of neuronal networks and an increase in the risk of seizures.

KCNC1 encodes $K_V3.1$, a member of the K_V3 subfamily that shows more positively shifted voltage-dependent activation and faster activation and deactivation rates than other K_V channels. A *de novo* mutation (R320H) in *KCNC1* has been identified in a patient with progressive myoclonic epilepsy, and shown to display LOF in a functional study [54]. $K_V3.1$ is preferentially expressed in fast-spiking inhibitory GABAergic interneurons and enables them to fire at high frequencies [55]. Lacking $K_V3.1$ function may impair the firing of fast-spiking GABAergic interneurons and subsequently result in hyperexcitability of the brain.

Both $K_V4.2$ (encoded by *KCND2*) and $K_V4.3$ (encoded by *KCND3*) are members of the K_V4 subfamily, which regulate the rate of low-frequency firing and control the backpropagation of action potentials into the dendritic tree [56]. A *de novo* mutation within *KCND2* (V404M) has been identified in a pair of twins with comorbidity of autism and epilepsy, showing GOF and profound impairment of closed-state inactivation [57]. A paternally-inherited truncated *KCND2* mutation (N587fsX1) has been found in a patient with temporal lobe epilepsy (incomplete penetrance, the father was not affected), which showed pLOF and a reduction of the inhibitory current contributing to aberrant neuronal excitability [58]. A *de novo* duplicated *KCND3* mutation (R293_F295dup) has been reported in a patient with generalized epilepsy and shows pLOF with a great depolarizing shift in the voltage-dependence of both $K_V4.3$ activation and inactivation [59]. Due to the limited data and a lack of a genotype (or funotype)-phenotype

Table 3 Mutations in the epilepsy-associated K^+ channel genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
KCNA2	EE	*de novo*	I263T, P405L	LOF with dominant-negative effect	[47]
			R297Q, L298F	GOF	
			L290R	Not available	
KCNB1	EE	*de novo*	R306C, S347R†, T374I†, V378A†, G379R†, G401R	LOF	[51–53]
			R312H, G381R, F416L	Not available	
KCNC1	PME	*de novo*	R320H	LOF with dominant-negative effect	[54]
KCND2	EE and autism	*de novo*	V404M	GOF	[57]
	TLE	Paternal#	N587fsX1	Destructive, pLOF	[58]
KCND3	GE	*de novo*	R293_F295dup	pLOF	[59]
KCNH2	Epilepsy with LQT2	AD	A429P	LOF	[61]
			Y493F	pLOF	
			c. 234-241del	Destructive, LOF	
		de novo	R863X	Destructive	[62]
		Unknown	I82T	LOF	[63]
KCNH5	EE	*de novo*	R327H	GOF	[64, 65]
KCNQ2	BFNS	AD	S247W, G271V, W344R, R353G	LOF	[70, 71, 79, 190, 191]
			E119G, S122L, A196V, L197P, R207W, R207Q, M208V, R214W, N258S, Y284C, A294G, A306T, R333Q, L351F, T359K, R553Q	pLOF	[67–78]
			L619R	GOF	[79]
			L351V, Y362C	Unchanged	[71]
			>15 Missense mutations	Not available	
			S247X, S399X, K537X, c.761_770del10insA	Destructive, LOF	[68, 70, 91]
			Q323X, R448X, V589X, P410fsX12, 867ins5bp	Destructive, pLOF	[70, 72, 75]
			R581X,W269X, 2 small insertions, 8 small deletions, 14 splicing	Destructive	
		de novo	D212G, R213W	pLOF	[89, 192]
			R333W	Not available	
			F304del	Destructive, LOF	[101]
			1 small deletion, 1 splicing site	Destructive	
	EE	*de novo*	A265P, T274M, G290D, A294V	LOF	[80, 81]
			S122L, A196V, I205V, M532W, R560W	pLOF	[81–84]
			R144Q, R201C, R201H	GOF	[86]
			>35 Missense mutations	Not available	
			W157X, 2 small indels, 1 small insertion,2 splicing	Destructive	
		Paternal#	R213Q	pLOF	[73, 82]

Table 3 continued

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
KCNQ3	BFNS	AD	I317T,W309R	LOF	[70, 90]
			E299K, D305G, G310V, N821S	pLOF	[75, 78, 91, 92]
			N468S	Unchanged	[75]
			R330C,R330H, G340V, R780C	Not available	
		de novo	R364H	Not available	
	BECTS	AD	A381V, P574S	Unchanged	[92]
	IE	Unknown	P574T	Not available	
	EE	AD	R330L	pLOF	[85]
		de novo	R230C	GOF	[86]
KCNV2	PS	Maternal#	R7K	GOF	[94]
	EE	Maternal#	M285R	GOF	
KCNMA1	GS and PD	AD	D434G	GOF	[96, 193]
KCNT1	ADNFLE	AD	G288S, R398Q,Y796H, R928C	GOF	[97, 100]
		de novo	M896I	GOF	[97, 105]
	EE	*de novo*	G288S,R398Q, R428Q, R474H, M516V, K629N, I760M, Y796H, P924L, A934T	GOF	[99, 101–105]
			H257D, R262Q, Q270E, V271F, P409S, R428Q, R429C, R429H, R474C, A477T, K629E, M896K, R933G, R950Q	Not available	
		Paternal‡	A966T (homozygote)	GOF	[194]

AD, autosomal dominant; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; AR, autosomal recessive; BECTS, benign epilepsy of childhood with centrotemporal spikes; BFNS, benign familial neonatal seizures; EE, epileptic encephalopathy; GE, generalized epilepsy; GS, general seizure; IE, idiopathic epilepsy; LQT2, long-QT syndrome type 2; PD, paroxysmal dyskinesia; PME, progressive myoclonic epilepsy; PS, partial seizure; TLE, temporal lobe epilepsy.

† Mutated channel lost K^+ selectivity and increased permeability to other positive and negative ions.

Incomplete penetrance; transmitter not affected.

‡ Patient had paternal isodisomy for chromosome 9; father not affected.

correlation, it is hard to define the association between K_V4 and epilepsy.

KCNH2 (also known as *hERG*) encodes $K_V11.1$ that is widely expressed in the human brain and heart. In the brain, $K_V11.1$ regulates neuronal firing and modulates the excitability of GABAergic and dopaminergic neurons [60]. *KCNH2* mutants were initially reported to be associated with long-QT syndrome type 2 (LQT2). To date, five mutations have been identified in patients with LQT2 and variable seizures. Functional analyses have shown LOF in all mutations [61–63], suggesting that LOF of $K_V11.1$ may increase the risk of epilepsy.

KCNH5 encodes $K_V10.2$ that is selectively expressed in interneurons localized to layer IV of the cerebral cortex in multiple areas, especially in numerous excitatory interneurons [64]. A patient with EE and multiple neurodevelopmental deficits has been reported to carry a *de novo* R327H mutation that confers a GOF change in the $K_V10.2$ channel [64, 65]. Since layer IV contains both excitatory and inhibitory interneurons [65], it is hard to estimate the effect of this $K_V10.2$ mutant on epilepsy.

KCNQ2 encodes $K_V7.2$ and *KCNQ3* encodes $K_V7.3$. K_V7 channels mediate low-threshold, slowly-activating, non-inactivating muscarinic currents [66]. Opening of homogeneous $K_V7.2$ or heterogeneous $K_V7.2/K_V7.3$ complexes inhibits initiation of the action potential and thus suppresses neuronal excitability [66]. Mutations in *KCNQ2* were initially identified in patients with benign familial neonatal seizures (BFNS). Functional studies have illustrated LOF or pLOF in a majority of mutants [67–78], GOF in one mutation (L619R) [79], and no significant change in two mutations (L351V and Y362C) [71]. Mutations in *KCNQ2* have also been identified in patients with EEs. Nine mutations demonstrate LOF or pLOF [80–85], while three (R144Q, R201H, and R201C) demonstrate GOF by stabilizing the activated state of the channels [86]. Mutations A196V and S122L have been identified in both benign BFNS and intractable EE [68, 69, 83, 84]. Mice expressing LOF mutant $K_V7.2$ channels display spontaneous seizures, behavioral hyperactivity, and increased hippocampal neuronal excitability and cell death [87]. A 25% reduction in the muscarinic current amplitude is sufficient to cause electrical hyperexcitability and leads to neonatal/infantile epilepsy in humans [78, 88]. Therefore, LOF of $K_V7.2$ leads to neuronal hyperexcitability and induces epileptogenesis. The EE-related R213Q mutation causes significantly more evident kinetic alterations than the BFNS-related R213W mutation [89], suggesting a potential genotype-phenotype correlation. The role of K_V7 GOF mutants in epileptogenesis is still under debate [79, 86]. Similarly, mutations in *KCNQ3* have been identified in patients with BFNS and mainly show LOF or pLOF [70, 75, 78, 90–92]. Another two *de novo KCNQ3* mutations have been identified in patients with EEs and each displays pLOF or GOF [85, 86].

KCNV2 encodes $K_V8.2$, which is electrophysiologically silent when expressed as a homotetramer. However, when assembled with K_V2 subunits, $K_V8.2$ significantly reduces the membrane expression of heterotetrameric channels and suppresses K_V2 currents [93]. The $K_V8.2$ and $K_V2.1$ subunits show a remarkable regional overlap in their CNS expression patterns [60]. Two mutations in *KCNV2*, R7K and M285R, have been identified in patients with partial seizures and EE, respectively [94]. They show GOF and enhanced Kv8.2-mediated suppression of $K_V2.1$ currents, subsequently reducing $K_V2.1$ currents and leading to epilepsy. The M285R mutant, which was identified in a patient with EE, also causes defects of $K_V2.1$ activation kinetics [94], potentially explaining the more severe phenotype.

KCNMA1 encodes the α-subunit of large-conductance Ca^{2+}-activated $K_{Ca1.1}$ channels. $K_{Ca1.1}$ is predominantly expressed in the axons and presynaptic terminals of neurons and promotes high-frequency firing [95]. A GOF mutation in *KCNMA1* has been detected in a large family with generalized epilepsy and paroxysmal dyskinesia [96]. The enhanced Ca^{2+}-activated K^+ current (BK current) increases the firing rate and spontaneous non-convulsive seizures in mice [96]. Thus it is possible that GOF of $K_{Ca1.1}$ increases the BK current and enables faster re-priming (removal of inactivation) of Na^+ channels, leading to hyperexcitability.

KCNT1 encodes the α-subunit of the Na^+-activated channel $K_{Ca4.1}$ (also known as Slack, KCNT1, or Slo2.2), which is highly expressed in many regions of the brain, and significantly found in neurons of the frontal cortex [97]. The precise function of homotetrameric $K_{Ca4.1}$ channels is unclear. Functional heterotetrameric channels consisting of $K_{Ca4.1}$ and $K_{Ca4.2}$ (encoded by *KCNT2*) subunits contribute to the delayed outward current I_{KNa}, which helps to modulate neuronal excitability and adaptability in response to high-frequency stimulation [98]. Mutations in *KCNT1* have been found in ADNFLE and EEs (especially epilepsy of infancy with migrating focal seizures). Two mutations (G288S and R398Q) have been identified in both ADNFLE and EE patients. All known functional consequences of *KCNT1* mutations show a strong GOF effect [97, 99–105]. Although the actual mechanisms by which GOF mutations lead to neuronal hyperexcitability are uncertain, *KCNT1* could be confirmed as an epilepsy gene when clinical evidence is taken into account.

Calcium Channel Genes

Voltage-gated Ca^{2+} (Ca_V) channels conduct an inward Ca^{2+} current after depolarization, mediate action potential

Table 4 Mutations in epilepsy-associated Ca^{2+}-channel genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
CACNA1A	EE	*de novo*	E101Q, A712T, A713T, S1373L, A1511S	Not available	
	IGE	Unknown	R477H, R1967Q	Not available	
			Q1397X	Destructive	
CACNA1H	CAE	Inherited	F161L, E282K, C456S, G499S, P648L, G773D, R788C, V831M, A876T, T1606M, R1892H	GOF	[109–112]
			T1733A†	pLOF	
			R744Q, A748V, G784S,G848S, Q1264H, D1463N	Unchanged	[109, 111]
			P314S, P492S, H515Y, Q1264H	Not available	
	MAE	Inherited	G983S	LOF	[112]
	IGE	Inherited	R788C, T1606M, A1705T#	GOF	[112]
			A1059T	pLOF	
			E1170K	Unchanged	
		Unknown	A480T, P618L, G755D, E1170K	Not available	
			V621fsX33	Destructive	
CACNA2D2	EE	AR	L1040P (homozygous)	pLOF	[113]
			N432fsX (homozygous)	Destructive	
CACNB4	JME	AD	R482X	Destructive, LOF	[115]
	IGE	AD	C104F	LOF	[115]

AD, autosomal dominant; AR, autosomal recessive; CAE, childhood absence epilepsy; EE, epileptic encephalopathy; IGE, idiopathic generalized epilepsy; JME, juvenile myoclonic epilepsy; MAE, myoclonic-astatic epilepsy.

†Not segregated with epilepsy in the two affected siblings of a CAE family. #A1705T co-segregates with R788C in all carriers.

firing and membrane oscillations, and thus have widespread effects on neuronal excitability [106]. Each Ca_V channel consists of one principal α1 subunit, which forms the pore and defines the channel type, and modulates β, α2δ, and possibly γ subunits. *CACNA1A*, *CACNA1H*, *CACNA2D2*, and *CACNB4* are associated with epilepsies (Table 4).

CACNA1A encodes the α1 subunit of $Ca_V2.1$, forming a P/Q-type voltage-gated Ca^{2+} channel. Mutations in *CACNA1A* have been identified in an IGE cohort [107]. One recent study demonstrated *de novo CACNA1A* mutations in patients with EEs [108]. Functional studies on these mutants have not been performed.

CACNA1H encodes the α1 subunit of $Ca_V3.2$, a member of the Ca_V3 subfamily. Ca_V3 channels are highly expressed in thalamic neurons, conduct low-voltage activated T-type (transient) Ca^{2+} currents, and play roles in circadian rhythms. Twenty-two mutations in *CACNA1H* have been identified in patients with childhood absence epilepsy (CAE), and most of them alter the channel kinetics. Based on functional studies and computer simulation, 11 mutations have been shown or predicted to display GOF [109–112], while six have been shown or predicted to cause no alteration in channel function [109, 111]. A GOF mutation (R1584P) in *Cacna1h* has been identified in the "Genetic Absence Epilepsy Rats of Strasbourg" model, and the T-type currents increase with age, mirroring the temporal profile of epilepsy development [106]. In addition, mutations in *CACNA1H* have been identified in patients with other types of idiopathic generalized epilepsy (IGE), and the changes in channel function are similar to CAE-related mutations [112]. These results suggest that GOF of *CACNA1H* in humans may increase neuronal firing by decreasing the threshold for rebound burst firing and thus lead to hyperexcitability. LOF has occasionally been identified in IGE- and CAE-related mutations, but the clinical and experimental data are insufficient to ascertain the pathogenicity of these mutants.

CACNA2D2 encodes the α2δ-2 subunit, which co-assembles with the α1 subunit of high-voltage P/Q-type Ca^{2+} channels ($Ca_V2.1$) in the cerebellum and hippocampus. α2δ-2 increases the whole-cell Ca^{2+} current amplitude and accelerates inactivation. Two homozygous mutations (L1040P and N432fsX) in *CACNA2D2* have been identified in patients with EE. Functional analysis of L1040P showed pLOF [113]. *Entla* mice carrying a nonfunctional α2δ-2 subunit show absence seizures [114]. Deficient α2δ-2 function in humans is expected to slow the inactivation of $Ca_V2.1$, thus increasing the action of Ca_V and leading to epileptogenesis.

Table 5 Mutations of epilepsy-associated Cl^- channel genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
CLCN2	JME	Paternal†	R235Q	pLOF	[119]
	GTCS	Paternal†	R644C#	Unchanged	
		Unknown	R577Q	pLOF	
	IGE	Unknown	S719L	Not available	
	IE	Paternal†	G715E	pLOF	[120]‡
		Unknown	G44R, R73H, F82L, S758N, A760V	Not available	
			W570X	Destructive	
CLCN4	EE	*de novo*	L221P, V275M, S534L, G544R§	LOF	[64, 122]
			A555V, R718W	pLOF	[122]
			D15N	Unchanged	[122]
		Inherited	V212G, G731R	LOF	[122]
			G78S, L221V, V536M	pLOF	
			D15fsX18, I626fsX135, intron9+5G>A, 1 intragenic copy number deletion	Destructive	

EE, epileptic encephalopathy; GTCS, generalized tonic-clonic seizure; IE, idiopathic epilepsy; IGE, idiopathic generalized epilepsy; JME, juvenile myoclonic epilepsy.

† Incomplete penetrance; transmitter not affected.

Also found in five Indian controls (5/89, 2.8%), but not in Caucasian (386) and North African (263) controls.

‡ Two mutations with false family data were not included. §Two unrelated carriers had different nucleotide substitutions (c.1630G > A and c.1630G > C).

CACNB4 encodes the β4 subunit, an auxiliary subunit of $Ca_V2.1$ [115]. The β4 subunit may enhance trafficking and expression of the α1 subunit, shift the channel activation to more hyperpolarized potentials, and increase the channel-opening probability [106]. One truncated mutation (R482X) has been identified in a family with juvenile myoclonic epilepsy (JME), and one missense mutation (C104F) has been identified in two families with IGE. A functional study has revealed that C104F exerts an effect similar to the destructive mutation R482X and increases Ca^{2+} currents [115], probably due to the impaired ability to shift channel activation toward hyperpolarized potentials. *Cacnb4* knock-out mice exhibit a "lethargic" phenotype of nonconvulsive seizures, ataxia, and dyskinesias [116]. Specific β4 subunit isoforms have been observed to accumulate in the nucleus, but are suspected to be involved in the pathogenesis of phenotypes other than epilepsy [117]. The involvement of β4 mutants in epileptogenesis is still unclear.

To date, clinical and experimental evidence suggests that Ca^{2+} channels are implicated in epilepsy. However, the distinct roles of Ca^{2+} channels in epilepsy phenotypes warrant further clarification.

Chloride Channel Genes

Cl^- channels (CLCs) are ubiquitously distributed and fulfill diverse functions. The CLC family encompasses nine human proteins, which are divided into two functional subgroups: Cl^- channels (CLC channels) and chloride-proton (Cl^-/H^+) exchangers (CLC exchangers) [118]. The CLC channels are located in the membranes of excitable and epithelial cells and regulate membrane excitability and the transport of electrolytes, water, and nutrients; the CLC exchangers are mainly expressed intracellularly and may play housekeeping roles [118]. *CLCN2* and *CLCN4* are reported to be associated with epilepsy (Table 5).

CLCN2 encodes CLC-2, which is an inwardly rectifying channel that opens very slowly upon hyperpolarization. Besides the voltage changes, CLC-2 can be activated by cell swelling. Eleven *CLCN2* mutations have been reported to be related to idiopathic epilepsy. Among four mutations with functional studies, three showed pLOF [119, 120]. *Clcn2* knock-out mice develop leukodystrophy with vacuoles slowly appearing in the myelin sheaths of central axons [121], but the precise function of CLC-2 in human neurons remains poorly understood.

CLCN4 encodes CLC-4, which is a strongly voltage-dependent $2Cl^-/H^+$ exchanger and is expressed widely. The functions of CLC-4 include endosomal acidification and trafficking. *CLCN4* mutations have been identified in patients with EE and X-linked intellectual disability. Functional analyses have mainly shown LOF or pLOF [64, 122, 123]. *Clcn4* depletion in cultured rodent neurons causes less-branched dendrites and axons [123, 124].

γ-Aminobutyric Acid Type A Receptor ($GABA_A$ Receptor) Genes

The $GABA_A$ receptors are a group of ligand-gated Cl^- channels. In the human brain, most $GABA_A$ receptors are heteropentamers consisting of two α(1-6), two β(1–3), and one γ(1–3) or δ subunits [125], of which the α1β2γ2 receptor is the most common [126]. Heterodimers (formed by an α and a β subunit) and homopentamers (formed by five β3 subunits) exist in small amounts under physiological conditions. By allowing Cl^- influx through its pore, the $GABA_A$ receptor mediates phasic (synaptic) or tonic (perisynaptic) inhibitory transmission in the brain, leading to hyperpolarization [127]. Epilepsy-associated $GABA_A$ receptor genes are listed in Table 6.

GABRA1 encodes an α1 subunit that is essential for the initiation of GABA-evoked potentials. Mutations in *GABRA1* were initially identified in a large family with JME [128], and the phenotypic spectrum was later expanded to other IGEs including CAE [129] and GEFS+ [130], as well as EEs [130–133]. Functional studies have demonstrated that all the examined mutants displayed LOF or pLOF [128–130, 132, 134] with trafficking impairment causing retention in the endoplasmic reticulum [127]. Heterozygous *Gabra1*-knock-out mice display spike-wave discharges and absence-like seizures [135]. *GABRA6* encodes an α6 subunit. A pLOF mutation in *GABRA6* has also been identified in a patient with CAE and disruption of the α6 subunit is associated with δ subunit dysfunction [136, 137].

GABRB1, *GABRB2*, and *GABRB3* encode β1, β2, and β3 subunits, respectively. The β subunits are expressed predominantly in human brain with temporal specificity [138]. The expression of β1 is the most abundant after birth, and then gradually decreases and maintains a lower level in mature neurons. In contrast, the expression of β2 changes more dynamically with development, with the highest expression during childhood and adolescence; the highest β3 level also occurs in early development but remains relatively constant. Two *de novo* mutations in each *GABRB1* and *GABRB2* have been identified in patients with EEs. Their functional analyses showed LOF or pLOF consequences. Mutations in *GABRB3* have been identified in patients with CAE and EEs and present LOF or pLOF [139–141] on current density. Besides decreased current, the CAE-related mutations in *GABRB3* also result in hyperglycosylation [141], which might further disturb channel function.

GABRG2 encodes the γ2 subunit that is critical for receptor trafficking, clustering, synaptic maintenance, and current kinetic properties [126]. Twenty-six mutations in *GABRG2* have been reported in a broad spectrum of epilepsies. Functional studies have illustrated LOF or pLOF [83, 126, 142–151] of these mutations, accompanied commonly by loss or reduction of γ2 subunit protein surface expression [152]. Similarly, the loss of γ2 in heterozygous *Gabrg2*-knock-out DBA/2J mice results in absence seizures [153], while heterozygous *Gabrg2* Q390X-knock-in C57/BL/6J mice display more severe phenotypes, with spontaneous generalized tonic-clonic seizures and probable sudden unexpected death in epilepsy [154]. Recent studies suggest that cellular homeostasis is also disturbed by γ2 mutations [152].

GABRD encodes the δ subunit. The δ-containing $GABA_A$ receptors exhibit preferential sensitivity to extracellular GABA concentrations [155], mediating tonic inhibition. Three mutations have been identified in patients with GEFS+ or JME. Functional analysis has shown pLOF in two mutations (E177A and R220H) and no changes in one (R220C) [156].

The mutations in the $GABA_A$ receptor genes identified in human epilepsies illustrate important relationships between $GABA_A$ receptor function and epileptogenesis. It seems that the LOF or pLOF effects of $GABA_A$ receptor genes are common mechanisms underlying the epilepsies caused by $GABA_A$ gene mutations. Hints of such a connection also come from knock-out studies of $GABA_A$ genes, in which the loss of $GABA_A$ function in animals leads to epilepsy-related activities. It is conceivable that impaired function of $GABA_A$ receptors would decrease inhibitory effects and lead to impaired coupling of neuronal excitation and inhibition; however, the precise pathogenic mechanism of $GABA_A$ receptor gene mutations remains to be clarified.

N-Methyl-D-Aspartate Receptor (NMDAR) Genes

NMDARs are cation channels that are activated by the excitatory neurotransmitter glutamate. NMDARs play roles in excitatory synaptic transmission, plasticity, and excitotoxicity of the CNS [157]. An NMDAR is commonly a bi-heterotetrameric or tri-heterotetrameric channel, consisting of two obligatory GluN1 subunits and two auxiliary GluN2(A-D) or GluN3(A,B) subunits. Mutations of NMDAR subunits are associated with epilepsy and other neurodevelopmental phenotypes (Table 7).

GRIN1 encodes the ubiquitous GluN1 subunit that binds glycine during activation of NMDARs. *GRIN1* mutations have been identified in patients with profound developmental delay and severe intellectual disability [158]. A total of 13 mutations have been associated with epilepsy. Functional analyses of seven mutations suggest prevalent pLOF or LOF in five (Q556X, S560dup, Y647S, G815R, and G827R), although the other two (R645S and R844C) did not show any functional alterations [158, 159]. Homozygous mutation (Q556X) carriers present more

Table 6 Mutations of epilepsy-associated $GABA_A$ receptor genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
GABRA1	CAE	*de novo*	S326fsX328	Destructive, LOF	[129]
	JME	AD	F104C, A322D	pLOF	[128, 130]
		Unknown	c.-248+1 G>T	Destructive	
	GEFS+	Paternal†	V74I	Not available	
	IGE	AD	D219N	pLOF	[134]
			K353delins18X	Destructive, LOF	[134]
		Maternal†	c.256-8 T>G	Destructive	
		Unknown	T20I, L215V, D219N	Not available	
	MAE	*de novo*	K306T	pLOF	[130]
	SME	*de novo*	S76R, G251S, K306T	pLOF	[130, 132]
			R112Q, L146M, R214H, T292I	Not available	
	EE	*de novo*	S76R, R214H	pLOF	[130]
			R112Q, N115D, G251D, P260L, M263I, M263T, V287L, T289P	Not available	
			K401fsX25	Destructive	
		Unknown	T289A	Not available	
GABRA6	CAE	Paternal†	R46W	pLOF	[136, 137]
GABRB1	IS	*de novo*	F246S	pLOF	[139]
	EE	*de novo*	T287I	Not available	
GABRB2	GTCS	*de novo*	M79T	Not available	
	EE	*de novo*	T287P	LOF	[140]
GABRB3	CAE	AD	P11S, S15F, G32R	pLOF	[141]
	IS	*de novo*	N110D	pLOF	[131, 139]
	LGS	*de novo*	D120N, E180G, Y302C	LOF	
	EE	*de novo*	L170R, Y182F, Q249K, L256Q, T287I, A305V, A305T	Not available	
GABRG2	FS	AD	R177G	pLOF	[142]
			R136X	Destructive, pLOF	[143, 146]
			V462fsX33	Destructive	
	BECTS	*de novo*	R323Q	pLOF	[144]
		AD	c.549-3T>G	Destructive	
	CAE with FS	AD	c.769+2T>G	Destructive, pLOF	[145]
	GEFS+	AD	P83S, K328M	LOF	[147, 150]
			R82Q	pLOF	[150]
			M199V	Not available	
		Unknown	R304K, R363Q	Not available	
		AD	Q390X	Destructive, LOF	[146, 148]
			W429X, Y444fsX51	Destructive, pLOF	[146, 151]
			E402fsX3	Destructive	
	GTCS	Unknown	N79S	Unchanged	[150]
	IGE	AD	G257R	Unchanged#	[144]
			P59fsX12	Destructive	
	EE	*de novo*	A106T, I107T, P282S, R323W, R323Q, F343L	pLOF	[83, 126]
		Paternal†	Q40X	Destructive, pLOF	[149]
GABRD	GEFS+	AD	E177A	pLOF	[156]
			R220C	Unchanged	
	JME	AD	R220H	pLOF	[156]

BECTS, benign epilepsy of childhood with centrotemporal spikes; CAE, childhood absence epilepsy; EE, epileptic encephalopathy; FS, febrile seizure; GEFS+, generalized epilepsy with febrile seizures plus; GTCS, generalized tonic-clonic seizure; IGE, idiopathic generalized epilepsy; IS, infantile spasms; JME, juvenile myoclonic epilepsy; LGS, Lennox-Gastaut syndrome; MAE, myoclonic-astatic epilepsy; SME, severe myoclonic epilepsy.

† Incomplete penetrance; transmitter not affected.

Reduced surface expression.

Table 7 Mutations in epilepsy-associated NMDAR genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
GRIN1	FS	*de novo*	R645S	Unchanged	[158]
			S549R	Not available	
	EE	*de novo*	G827R	LOF	[158]
			Y647S, G815R	pLOF	[158]
			R844C	Unchanged	[158]
			R417S, D552E†. M641I, N650K, G815V	Not available	
			S560dup	Destructive, LOF	[159]
	Fatal EE	Inherited	Q556X (homozygous)	Destructive, LOF	[158]
GRIN2A	IFE	Unknown	A243V	GOF	[162]
			A290V, G295S, R370W, K669N, P699S, E714K, A727T, K772E, N976S, A1276G	Not available	
			W198fsX, Ser547del	Destructive	
		Paternal#	P183I, V734L, I814T	Not available	
			L779SfsX5	Destructive	
		Maternal#	I184S, C231Y, G483R, R504W, M705V, D933N	Not available	
			P31SfsX107	Destructive	
		AD	R518H	GOF	[163]
			M1T, P79R, D731N, A716T, I904F	Not available	
			V529TfsX22, R681fsX, Y1387fsX	Destructive	
		de novo	F652V	GOF	[164]
			C436R, A548T, I694T, CNV del	Not available	
	FE and/or GE	AD	T531M	GOF	[165]
			D1251N	Not available	
			Y943fsX, L334X, Q218fsX, c.1007+1G>A, c.1123-2A>G, c.2007+1G>A, 3 chromosomal abnormalities	Destructive	
		de novo	M817V	Not available	
			2 chromosomal abnormalities	Destructive	
		Unknown	V506A	Not available	
			c.1007+1G>T, 1 chromosomal abnormality	Destructive	
	EE	*de novo*	N615K, L812M	GOF	[166, 167]
	Severe unclassified	*de novo*	P552R	Not available	
		Unknown	L649V	Not available	
GRIN2B	TLE	Paternal#	E47Q	GOF	[168]
		Unknown	E370K	Not available	
	FE	*de novo*	R540H	GOF	[169]
	EE	*de novo*	N615I, V618Q	GOF	[169]
			C461K	Not available	
		Paternal#	c.2011-5_2011-4delTC	Destructive	
	Unclassified epilepsy and ID	*de novo*	1 chromosomal deletion	Destructive	
		Unknown	1 chromosomal deletion	Destructive	
GRIN2D	EE	*de novo*	V667I	GOF	[170]

BECTS, benign epilepsy of childhood with centrotemporal spikes; EE, epileptic encephalopathy; FE, focal epilepsy; GE, generalized epilepsy; ID, intellectual disability; IFE, idiopathic focal epilepsy; IGE, idiopathic generalized epilepsy; TLE, temporal lobe epilepsy.

† Two unrelated carriers had different nucleotide substitutions (c.1656C > A and c.1656 C > G).

Incomplete penetrance; transmitter not affected.

severe clinical phenotypes (fatal EE), while homozygous targeted knock-out mice display abnormal glutamate-mediated receptor currents and result in perinatal lethality. These findings indicate that the GluN1 subunit plays an essential role in neurodevelopment. It is therefore possible that dysfunction of the GluN1 subunit may lead to abnormal neurodevelopment as well as epileptogenesis.

GRIN2A, *GRIN2B*, and *GRIN2D*, which encode the GluN2A, GluN2B, and GluN2D subunits, respectively, have been associated with epilepsy. The GluN2 subunits have a common binding site with L-glutamate for activation of NMDARs, but show differential spatial and temporal expression patterns throughout the CNS. *GRIN2A* is mainly expressed in the hippocampus and cerebral cortex at infant and adult stages [157]. In contrast, *GRIN2B* is expressed in the whole brain during the embryonic period and in the forebrain after adulthood [160]. The expression of *GRIN2D* is mostly in the limbic system and interneurons in cortico-limbic regions during embryonic stages and is reduced after birth [161].

GRIN2A mutations have been mainly identified in patients with focal epilepsy (FE) and speech disorder, typically in those with Rolandic spikes. Recently, missense mutations have been identified in patients with other phenotypes like EEs or severe unclassified epilepsy. From the published data, there is no distinct relationship between genotype and the severity of epilepsy. Missense mutations of *GRIN2A,* four (A243V, R518H, T531M, and F652V) from FE patients and two (N615K and L812M) from EE patients, have presented a consequence of GOF [162–167]. These GOF mutants display increased activation at low concentrations of agonists and extended durations of channel open and closed states, thus leading to an excessive excitatory drive and epileptogenesis. However, destructive *GRIN2A* mutations have also been identified in patients within the spectrum of epilepsies, including FE and EE. It remains to be clarified how the destructive mutations impact the function of NMDARs and lead to epilepsy. Considering that GluN2A is not a ubiquitous subunit, it is possible that the destructive GluN2A subunit is substituted by other functionally different subunits such as other GluN2 or GluN3, and thus entails functional changes of NMDARs.

Nine *GRIN2B* mutations have been identified in patients with epilepsies, including idiopathic focal epilepsy, temporal lobe epilepsy, and EEs. Functional analyses on four missense mutations (E47Q, R540H, N615I, and V618Q) have shown that they all lead to GOF [168, 169]. *GRIN2B* mutants have a spectrum of genotypes and phenotypes similar to *GRIN2A*, suggesting a similar mechanism in pathogenicity.

One *de novo* mutation in *GRIN2D* (V667I) has been identified in two unrelated patients with EE [170]. Functional analysis has shown a GOF effect with increased current. Transfection of cultured neurons with the V667I mutant causes dendritic swelling and neuronal death, suggestive of excitotoxicity mediated by NMDAR over-activation.

Neuronal Nicotinic Receptor (nAChR) Genes

The nAChRs are a family of pentameric cation channels that are activated by acetylcholine, producing post-synaptic excitation and neurotransmitter release. Sixteen genes encoding nAChRs have been identified in humans. Four nAChR genes, *CHRNA2*, *CHRNA4*, *CHRNA7*, and *CHRNB2*, have been associated with epilepsy (Table 8).

CHRNA4 was the first identified epilepsy gene; it encodes the α4 subunit of nAChRs. The α4 subunit is a component of the high-affinity and slowly desensitizing heteropentamer α4β2*, which is one of the two most common nAChRs in the human brain. To date, six *CHRNA4* mutations have been identified in nocturnal frontal lobe epilepsy, five in familial cases and one in a sporadic case. The mutations in functional studies commonly display GOF [7, 171, 172]. Several additional variations with undefined pathogenicity have been reported in cases of ADNFLE [173] and other epilepsy phenotypes [174].

CHRNA2 encodes the α2 subunit that composes a heteromeric nAChR with both β2 and β4 subunits. Two *CHRNA2* mutations (I279N and I297F) have been reported in two unrelated ADNFLE families. Functional studies have shown GOF of the I279N mutation [175] and pLOF of the I297F mutation [176]. Recently, one mutation (R376W) was identified in a family with benign familial infantile seizures [177].

CHRNB2 encodes the β2 subunit that participates in forming the heteropentamers α4β2* and α2β2β4. The precise function of β2 subunit is unclear. Genetic deletion of the β2 subunit in mice leads to a reduction of dendritic spine density in pyramidal neurons in pre-limbic and infra-limbic areas [178]. Five mutations have been identified in patients with ADNFLE and another two in an IGE cohort. GOF was found in three ADNFLE-related mutants (V287L, V287M, and L301V) [7, 171, 179].

CHRNA7 encodes the α7 subunit that composes a low-affinity and quickly-desensitizing homopentamer. This homopentamer (i.e. $(\alpha7)_5$) is also a common type of nAChR in human thalamus and isocortex. Four chromosome deletions and one chromosome triplication (all including entire *CHRNA7*) have been identified in patients with IGE. Since *CHRNA7* deletion and duplication can be found in affected probands as well as in asymptomatic parents and healthy controls [180], their pathogenicities are uncertain.

Table 8 Mutations in epilepsy-associated nAChR genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
CHRNA2	ADNFLE	AD	I279N	GOF	[175]
			I297F	pLOF	[176]
	BFIS	AD	R376W	Not available	
	IGE	Unknown	T47M	Not available	
CHRNA4	ADNFLE	AD	S280F, S284L, T293I	GOF	[7, 171, 172]
			I275F	Not available	
			c.870_872dupGCT	Destructive, GOF	[171]
	NFLE	Maternal†	R308H	Not available	
CHRNA7	IGE	Unknown	4 gross deletions and 1 gross insertion	Destructive	
CHRNB2	ADNFLE	AD	V287L, V287M, L301V	GOF	[7, 171, 179]
			V308A, I312M	Not available	
	IGE	AD	H35Q, F478L	Not available	

ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; BFIS, benign familial infantile seizures; IGE, idiopathic generalized epilepsy; NFLE, sporadic nocturnal frontal lobe epilepsy.

† Incomplete penetrance; transmitter not affected.

Heteromeric nAChRs regulate both excitatory and inhibitory transmission in the frontal cortex, and the delicate balance of excitation and inhibition is crucial for normal neuronal activity. For instance, GOF of nAChRs (by introducing *Chrna4*-S252F and *Chrna4*-L264ins in mice) produces abnormally strong GABA release from GABAergic cells and causes synchronization of pyramidal cells [181]. On the other hand, LOF of nAChRs (using dihydro-β-erythroidine to block heteromeric nAChRs in mice) also decreases feedback inhibition of pyramidal cells in the same GABAergic cells and causes hyperexcitability [182]. Due to the complexity of the functional interactions, each nAChR gene or mutant may have a distinct effect on epileptogenesis. Hence, the exact underlying mechanisms of epileptogenesis for nAChRs warrant further clarification.

Hyperpolarization-Activated Cyclic Nucleotide-Gated (HCN) Channel Genes

The HCN channels are a group of cation channels that are dually activated by voltage hyperpolarization and intracellular cAMP, conducting a mixed Na^+-K^+ inward current[183]. In neurons, the HCN channels are mainly activated by hyperpolarization and contribute to cellular excitability and plasticity. *HCN1* and *HCN2* are considered to be associated with epilepsy (Table 9).

HCN1 encodes the HCN1 channel that is predominantly expressed in dendrites in the neocortex and hippocampus. Six missense mutations have been identified in patients with EEs. Two mutations (S272P and R297T) showed LOF, while another three (S100F, H279Y, and D401H) showed GOF [184]. There is no significant funotype-phenotype relationship. *Hcn1*-knockout mice display increased excitability and sensitivity to convulsants [185], suggesting that LOF of HCN1 may lead to epileptogenesis.

HCN2 encodes the HCN2 channel that is expressed evenly in the brain and relatively abundant in the thalamus. Three missense mutations have been identified in patients with FS and IGE. Functional studies have shown divergent effects. An FS-related mutation (S126L) shows GOF with faster kinetics at higher temperatures, indicating neuronal hyperexcitability during hyperthermia [186]. An IGE-related mutation (E515K) shows LOF with a reduced threshold of action potential firing and strongly increased excitability and firing frequency in rat primary cortical neurons [187]. The etiology of HCN2 mutants remains unclear.

Discussion

We systematically reviewed 41 ion channel genes that have been associated with epilepsies and analyzed their genotypes, funotypes, and phenotypes. These data are expected to provide clues in evaluating the pathogenic potential of these genes and understanding the underlying mechanism of epilepsy.

We have summarized the funotypes of mutations (Table 10). Genes with mutations featuring LOF include *SCN1B*, *KCNB1*, *KCNH2*, *KCNQ2*, *KCNQ3*, *CLCN2*, *CLCN4*, *GRIN1*, *GABRA1*, *GABRB3*, and *GABRG2*, in which both destructive and missense mutations are potentially pathogenic. Genes with mutations featuring GOF

Table 9 Mutations in epilepsy-associated HCN channel genes and their functional effects.

Gene	Phenotype	Inheritance	Mutations	Functional alteration	Ref.
HCN1	EE	*de novo*	S272P, R297T	LOF	[184]
			S100F, H279Y, D401H	GOF	
		Unknown	G47V	Not available	
HCN2	FS	Maternal†	S126L	GOF, faster kinetics at higher temperature.	[186]
	IGE	Maternal†	R527Q#	Unchanged	[195]
		AR	E515K (homozygous)	LOF	[187]

EE, epileptic encephalopathy; FS, febrile seizure; IGE, idiopathic generalized epilepsy.

† Incomplete penetrance; transmitter not affected.

The R527Q substitution was absent in the other affected sibling in the same family.

Table 10 Summary of pathogenic funotypes of epilepsy-associated ion channel genes.

Main funotype	Confirmed by multiple sources	To be confirmed
LOF	*SCN1B*	*KCNC1*, *KCND3*
	KCNB1, *KCNH2*, *KCNQ2*†, *KCNQ3*†	*CACNA1A*, *CACNA2D2*, *CACNB2*
	CLCN2†, *CLCN4*†	*GABRA6*, *GABRB1*, *GABRB2*, *GABRD*
	GRIN1†, *GABRA1*, *GABRB3*, *GABRG2*†	*CHRNA7*
GOF	*SCN8A*†	*SCN9A*
	KCNT1	*KCNH5*, *KCNV2*, *KCNMA1*
	GRIN2A#, *GRIN2B*#	*GRIN2D*
	CHRNA4, *CHRNB2*	
Both LOF and GOF	*SCN1A* (with distinct funotype-phenotype correlation)	*SCN2A, SCN3A*
		KCNA2, *KCND2*
		CACNA1H
		CHRNA2
		HCN1, *HCN2*

† With a few exceptions.

Have destructive mutations without examination of whole channel functions.

include *SCN8A*, *KCNT1*, *GRIN2A*, *GRIN2B*, *CHRNA4*, and *CHRNB2*. Missense mutations are therefore potentially pathogenic in general. Genes like *GRIN2A* and *GRIN2B* seem to be exceptional, in that epilepsy-related mutations feature GOF but destructive mutations have also been identified. One possible explanation is that the destructive mutants could be replaced by other subunits of similar function and lead to GOF, since the subunits encoded by *GRIN2A* and *GRIN2B* are not ubiquitous. Several genes display diversified funotypes, among which a distinct funotype-phenotype correlation was found in *SCN1A*. These data suggest that the funotype should be an essential consideration in evaluating the pathogenicity of mutations. A distinct funotype or funotype-phenotype correlation helps in defining the pathogenic potential of a gene.

Most epilepsy-related mutations are heterozygous. Since the human genome is diploid, a heterozygous mutation is generally considered to be potentially pathogenic in a phenotype of dominant inheritance. Therefore, familial co-segregation information is crucial in evaluating their pathogenicities. However, such information is not always available, *e.g.*, the *de novo* mutations identified in epilepsy phenotypes in recent years. To evaluate the role of these mutations and the related genes in epilepsy, other aspects such as the correlations between genetic impairment and phenotypic severity, genetic knock-out/knock-in outcomes, and pathogenic mechanisms should be taken into consideration. For heterozygous mutations featuring LOF in functional studies, the correlations between impairment and phenotypic severity and the presentations of genetic knock-out animals would help to define whether the variations are pathogenic. The gene expression patterns and underlying pathogenic mechanisms are also essential considerations, especially for genes with mutations

featuring GOF and those with limited data or divergent functional changes.

Generally, epilepsies are caused by abnormal synchronized electrical discharges within the brain [60], and ion channels are believed to regulate brain excitability and play critical roles in epilepsies. However, each ion channel plays a unique role in the generation and modulation of neuronal excitability, and thus may be associated with a distinct epilepsy phenotype or phenotype spectrum with a distinct mechanism. Typically, *SCN1A* has been confirmed to be associated with epilepsy by the distinct phenotype spectrum of FS-related epilepsies; its unequal expression on excitatory pyramidal neurons and inhibitory interneurons is critical in epileptogenesis; and the heterogeneous and relatively local distribution of inhibitory interneurons explains the common partial seizures. Similarly, mutations of *KCNQ2* are associated with epilepsy commonly featuring an early onset; it has been confirmed that a 25% reduction in K_V7-mediated muscarinic current amplitude is sufficient to result in electrical hyperexcitability. *KCNT1* mutations cause a spectrum of focal epilepsies with intellectual disability. Although their precise mechanisms are unclear, all functionally examined mutations in *KCNT1* display a definite and strong GOF effect on channel properties. Functional alterations of ion channel genes may directly result in excessive excitability (e.g. GOF in NMDAR mutants) or insufficient inhibitory activity (e.g. LOF in $GABA_A$ receptor mutants) in the CNS. In contrast, the pathogenic mechanisms of some genes underlying epileptogenesis, such as *SCN1A*, *KCNC1*, and *CACNA2D2*, are much more complex.

Epilepsy is a complex entity with various phenotypes and phenotype-specific etiologies. Individuals affected by epilepsy may respond differently to anti-epileptic treatments. The updated discovery of epilepsy-associated ion channel genes provides insights into the underlying mechanisms of epileptogenesis and helps to identify novel therapeutic targets for the development of anti-epileptic drugs and individualized treatment.

Conclusions

We systemically reviewed the mutations, funotypes, and phenotype information of 41 epilepsy-associated ion channel genes. These genes are characterized by distinct phenotypes and pathogenic funotypes. The distinct funotypes or funotype-phenotype correlations suggest that funotype should be an essential consideration in evaluating the pathogenicity of mutations. We highlight the complexity of the underlying epileptogenic mechanisms of each ion channel gene. Besides direct contributions to excessive excitability or insufficient inhibition, the phenotypic explanations should be extended to the molecular and ontological mechanisms of the genes.

Acknowledgements Research work from our laboratory cited in this review was supported by the National Natural Science Foundation of China (81571273, 81571274, 81501124, 81271434, and 81301107), Omics-based precision medicine of epilepsy being entrusted by Key Research Project of the Ministry of Science and Technology of China (2016YFC0904400), the Natural Science Foundation of Guangdong Province, China (2014A030313489), Science and Technology Planning Projects of Guangdong Province, China (2012B031800404 and 2013B051000084), the Department of Education of Guangdong Province, China (2013CXZDA022, 2013KJCX0156, and 2012KJCX009), the Foundation for High-level Talents in Higher Education of Guangdong Province, China (2013-167), Yangcheng Scholar Research Projects of Guangzhou Municipal College (12A016S and 12A017G), and Science and Technology Projects of Guangzhou, Guangdong Province, China (2014J4100069, 201508020011, 201604020161, and 201607010002).

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