Evaluating the pathogenic potential of genes with de novo variants in epileptic encephalopathies

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Epileptic encephalopathies comprise a group of catastrophic epilepsies with heterogeneous genetic etiology. Although next-generation sequencing techniques can reveal a number of de novo variants in epileptic encephalopathies, evaluating the pathogenicity of these variants can be challenging. Determining the pathogenic potential of genes in epileptic encephalopathies is critical before evaluating the pathogenicity of variants identified in an individual. We reviewed de novo variants in epileptic encephalopathies, including their genotypes and functional consequences. We then evaluated the pathogenic potential of genes, with the following additional considerations: (1) recurrence of variants in unrelated cases, (2) information of previously defined phenotypes, and (3) data from genetic experimental studies. Genes related to epileptic encephalopathy revealed pathogenicity with distinct functional alterations, i.e., either a gain of function or loss of function in the majority; however, several genes warranted further study to confirm their pathogenic potential. Whether a gene was associated with distinct phenotype, the genotype (or functional alteration)-–phenotype correlation, and quantitative correlation between genetic impairment and phenotype severity were suggested to be specific evidence in determining the pathogenic role of genes. Data from epileptic encephalopathy-related genes would be helpful in outlining guidelines for evaluating the pathogenic potential of genes in other genetic disorders.

INTRODUCTION

Epileptic encephalopathies embody a group of age-dependent devastating syndromes, which can be characterized by refractory seizures, severe electroencephalography (EEG) abnormalities, and psychomotor development delay or decline. Approximately 40% of new-onset epilepsies before the age of 3 years can be attributed to epileptic encephalopathy,[1] for example, Ohtahara syndrome, West syndrome, and Dravet syndrome. Recent studies have demonstrated the important role of genetic determinants underlying epileptic encephalopathy.[2] Most epileptic encephalopathy cases are clinically sporadic, coinciding with frequently identified de novo variants.[2] Although a number of de novo genetic variants have been detected in patients with epileptic encephalopathy with the application of next-generation sequencing (NGS), the causality of these variants remains elusive. Recently, several standards and guidelines have been recommended for the interpretation of sequence variants.[3,4] Generally, the pathogenicity of sequence variants should be evaluated at the variant level, as well as at the gene level.[3] At the gene level, the association between a gene and the disease, i.e., the pathogenic potential of the gene, which refers to the disease-causing potential if a variant occurred in this gene, should be evaluated. Further, implications, such as the potential pathogenic genotype, functional alteration, and genic subregion effect[5] should also be assessed. Generally, assessment of gene-level implications is required before evaluating the pathogenicity of variants identified in an individual.[4] In most situations, however, it is hard to determine the pathogenic potential of a gene based on data from an individual study, especially for genes with de novo variants that lack familial information of cosegregation. In the present study, we reviewed de novo variants identified in patients with epileptic encephalopathy and evaluated the pathogenic potential of the genes, with the aim of providing a basis for assessment of the pathogenicity of variants in a clinical or genetic consulting practice. We further assessed the evidence for evaluating the pathogenic potential of epileptic encephalopathy-related genes, with an expectation of enabling the formulation of guidelines for evaluating the pathogenic potential of genes.

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STRATEGY OF LITERATURE SEARCH AND ANALYSIS

Relevant articles were identified with searches on PubMed until November 2017. According to the classification defined by the International League Against Epilepsy (1989, 2001, and 2010), the following epileptic encephalopathy syndromes were taken into account: early myoclonic encephalopathy, Ohtahara syndrome, West syndrome (or infantile spasms), Dravet syndrome (or severe myoclonic epilepsy in infancy), Lennox–Gastaut syndrome, Landau–Kleffner syndrome, epilepsy with myoclonic–atonic seizures (EMAS), epileptic encephalopathy with continuous spike-and-wave during sleep (ECSWS), and epilepsy of infancy with migrating focal seizures (EIMFS). Regarding the technique, studies that employed any of the three major NGS technologies, i.e., genome sequencing, exome sequencing, and targeted sequencing (restricted in some interesting candidate genes), were included. We searched literature on the PubMed database using two keywords, namely, a combination of each syndrome and NGS technological aspect, e.g., "epileptic encephalopathy" and "next-generation sequencing." Using the same strategy, we searched the Ovid database and cross-checked the findings with the results from PubMed. We also searched OMIM (https://www.ncbi.nlm.nih.gov/omim/) for epileptic encephalopathy–associated genes. ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and HGMD (http://www.hgmd.cf.ac.uk/ac/index.php) were consulted for any missed variants. To observe the frequencies of variant in different genes, studies on identification of variants in a given gene by conventional sequencing method were excluded (Supplementary Figure S1 online).

We analyzed the genotype, functional alteration (funotype - the type of functional consequences of a variant),[6] and phenotype (subtype of epileptic encephalopathy) of de novo gene variants identified in epileptic encephalopathy. Missense variants were further specified by functional alterations that were classified as gain of function (GOF), loss of function (LOF), which referred to the complete loss of function, or partial loss of function, which was used to denote mutants with residual activities, as outlined in our previous reports.[6,7] Conversely, the functional consequence of variants that caused gross protein malformations and mostly led to haploinsufficiency, including truncating variants (nonsense and frameshifting), splice-site variants, and those causing genomic rearrangement, was referred to as destructive variants.[7]

The pathogenic potential of each gene was evaluated with the following considerations: (1) recurrence of variants in unrelated cases; (2) information of previously defined phenotypes of the genes, including inheritance pattern, and the correlation between genetic impairment and clinic severity; and (3) data from genetic experimental studies. Evidence for evaluating the pathogenic potential of epileptic encephalopathy-related genes was analyzed, from which a guideline was outlined.

PATHOGENIC POTENTIAL OF THE GENES

An overview

In total, 151 initial publications identifying de novo gene variants in epileptic encephalopathy were retrieved, in which 134 genes and 704 de novo variants were reported.

Regarding replication, 644 de novo variants were identified in 74 genes, which were suggested to be associated with epileptic encephalopathy by variants identified in two or more unrelated cases in each gene (Supplementary Table S1 online). These included 37 genes that were validated by independent studies, as well as recurrent variants in unrelated cases within a cohort; the remaining genes were validated either by single cases in more than one independent study or recurrent cases in a single study. All these genes are expressed in the brain. Fifty-two of these genes were previously reported to be associated with brain dysfunction phenotypes, among which epilepsies were the most frequent (48/52). Details of the variants and their original references are summarized in Supplementary Tables S2–6 online. These genes were subjected to further evaluation of pathogenic potential in epileptic encephalopathy (Supplementary Figure S1 online).

For the remaining 60 genes, only one de novo variant was identified in a single case for each gene (Supplementary Table S7 online). Their plausible relevance to epileptic encephalopathy was generally based on previous data showing a possible association with epilepsy in the original studies. These genes are either epilepsy-causing, or epilepsy-related, or potentially epilepsy-associated.[8] Considering that each individual carries at least one de novo variant in the exome or approximately 100 de novo variants in the whole genome,[9] the pathogenic potential of these genes in epileptic encephalopathy was not evaluated further.

We summarized the variants and their genotype, functional alteration, and sub-phenotype of epileptic encephalopathy, referred to the previously defined phenotypes of these genes (Tables 1,2, Supplementary Tables S8, S9 online), and evaluated the association between each gene and the epileptic encephalopathy.

Sodium channel genes

SCN1A, *SCN2A*, and *SCN8A*, which encode the principal α subunits of sodium channels $Na_v1.1$, $Na_v1.2$, and $Na_v1.6$, respectively, have been suggested to be associated with epileptic encephalopathy, as evidenced by multiple sources (Table 1, Supplementary Table S2 online).

Variants in the *SCN1A* gene were initially identified in mild familial generalized epilepsy with febrile seizures plus,[10] and were later confirmed to be the major cause of severe Dravet syndrome,[6] a typical form of epileptic encephalopathy. Previous studies have demonstrated a close correlation between genotype (or funotype) and phenotype of *SCN1A* variants.[6] Using NGS technologies, 78 *SCN1A* de novo variants have been identified in 87 patients with epileptic encephalopathy (Table 1). Existing data confirmed that these variants were destructive or caused LOF. *SCN1A* de novo variants were also identified in 7.0% (8/115) of patients with

Table 1 Genotype, functional alteration, and phenotype of variants in ion channel genes in epileptic encephalopathies

Gene	Genotype (variant no.)	Funotype (variant no.)	EE phenotype (case no.)	Previously reported phenotype (inheritance)
Sodium channel				
SCN1A	Missense (42)	GOF (0)	/	GEFS+ (AD mostly)
		pLOF (2), LOF (2)	DS (6)	PEFS+ (AD or de novo), DS (de novo mostly)
		UD (38)	DS (18), EIMFS (5), LGS (4), uEE (14)	
	Truncating (26), splice (10)	Destructive (36)	DS (28), LGS (4), EMAS (1), uEE (7)	
SCN2A	Missense (53)	GOF (2)	OS (2), uEE (3)	BFNS (AD), uEE (de novo)
		pLOF (0)	/	
		UD (51)	OS (12), WS (9), EIMFS (1), LGS (1), uEE (39)	
	Truncating (2)	Destructive (2)	LGS (1), uEE (1)	
SCN8A	Missense (45)	GOF (8)	DS (1), uEE (21)	/
		pLOF (1), LOF (1)	uEE (2)	/
		UD (35)	DS (2), EIMFS (1), LGS (1), WS (1), uEE (36)	BFIS (AD)
	Truncating (1), splice (1)	Destructive (2)	uEE (2)	/
Potassium channel				
KCNA1	Missense (2)	UD (2)	uEE (2)	EA/myokymia[a] (AD)
KCNA2	Missense (12)	GOF (3)	uEE (8)	GE (de novo)
		G-LOF (3)	uEE (5)	/
		LOF (4)	DS (2), EMAS (1), uEE (4)	FE (de novo), HSP[a] (AD)
		UD (2)	uEE (2)	/
KCNB1	Missense (9)	LOF (6)	WS (1), uEE (7)	/
		UD (3)	uEE (4)	/
	Truncating (1)	Destructive (1)	WS (1)	/
KCNQ2	Missense (64)	GOF (4)	WS (5), EME (1), uEE (3)	/
		pLOF (4), LOF (3)	OS (2), WS (2), uEE (9)	BFNS/uEE (AD mostly)
		UD (53)	OS (14), WS (7), uEE (41)	BFNS (AD mostly)
	Truncating (2)	Destructive (2)	WS (1), uEE (1)	BFNS (AD mostly)
KCNT1	Missense (29)	GOF (10)	EIMFS (24), WS (2), uEE (3)	NFLE (AD mostly)
		UD (19)	EIMFS (13), uEE (6)	/
Calcium channel				
CACNA1A	Missense (5)	UD (5)	EIMFS (1), LGS (1), uEE (5)	EA[a] (AD), FHM[a] (AD)
NMDA receptor				
GRIN1	Missense (7)	pLOF (1), LOF (2)	WS (1), uEE (2)	Severe ID[a] (de novo)
		UD (4)	uEE (5)	Severe ID[a] (de novo)
GRIN2A	Missense (3)	GOF (1)	uEE (1)	FE (AD)
		LOF (1)	LKS (1), uEE (1)	FE (AD or de novo)
		UD (1)	uEE (2)	FE (AD or de novo)
	Truncating (0)	Destructive (0)	/	FE (AD mostly)
GRIN2B	Missense (12)	GOF (2)	WS (2)	FE (de novo)
		UD (10)	WS (7), LGS (1), uEE (2)	/
	Splice (1)	Destructive (1)	uEE (1)	/
GRIN2D	Missense (1)	GOF (1)	uEE (2)	/
Cyclic nucleotide-gated ion channel				
HCN1	Missense (7)	GOF (3)	uEE (3)	/
		LOF (2)	uEE (2)	/
		UD (2)	uEE (2)	IGE (unknown)

Variants in GABA-A receptor genes are summarized in Supplementary Table S 8 online, and all known functional alterations in these genes were LOF. Genes with pathogenic potential to be confirmed are not listed here. Details in Supplementary Table S2–4 online

AD autosomal dominant, *BFIS* benign familial infantile seizures, *BFNS* benign familial neonatal seizures, *DS* Dravet syndrome, *EA* episodic ataxia, *EE* epileptic encephalopathy, *EIMFS* epilepsy of infancy with migrating focal seizures, *EMAS* epilepsy with myoclonic–atonic seizures, EME early myoclonic encephalopathy, *FE* focal epilepsy, *FHM* familial hemiplegic migraine, *GE* generalized epilepsy, *GEFS+* generalized epilepsy with febrile seizures plus, *G-LOF* gain- and loss-of-function, *GOF* gain of function, *HSP* hereditary spastic paraplegia, *ID* intellectual disability, *IGE* idiopathic generalized epilepsies, *LGS* Lennox–Gastaut syndrome, *LKS* Landau–Kleffner syndrome, *LOF* loss of function, *NFLE* nocturnal frontal lobe epilepsy, *OS* Ohtahara syndrome, *PEFS+* partial epilepsy with febrile seizures plus, *pLOF* partial loss of function, *UD* functional alteration not determined missense, *uEE* unspecified epileptic encephalopathy, *WS* West syndrome
[a]Phenotype with seizures

Lennox–Gastaut syndrome and occasionally in other epileptic encephalopathies. These findings confirmed that LOF of $Na_v1.1$ is the main cause of Dravet syndrome and highlighted its possible role in other forms of epileptic encephalopathy.

SCN2A variants were first identified in families with benign familial neonatal–infantile seizures.[11] Recent large-scale sequencing initiatives have identified 55 de novo variants in 69 sporadic cases with epileptic encephalopathy (Table 1), including several hotspot variants. Most *SCN2A* variants were missense. However, both LOF and GOF were detected in mutants from either benign familial neonatal–infantile seizures or epileptic encephalopathy, with no distinct difference in functional alternation between them.[12] In the mouse model, heterozygotes for the *Scn2a* knockout did not show seizures.[13] Therefore, the functional alterations caused by heterozygous *SCN2A* variants, especially LOF variants, cannot explain their pathogenicity in epileptic encephalopathy.

SCN8A was a novel gene that was reported to be associated with epileptic encephalopathy, with the identification of 47 de novo variants (Table 1), which were all missense variants except two. The R850, R1617, A1650, and R1872 residues were four hot spots, with variants at these residues accounting for 34.3% (23/67) of patients with epileptic encephalopathy (Supplementary Table S2 online). Electrophysiological

alterations were determined in 10 mutants, of which 8 showed a GOF effect, including R1617 and R1872. These findings suggest that *SCN8A* variants with GOF are potentially pathogenic. Two mutants displayed LOF or partial LOF effect. However, *Scn8a*-null heterozygotes mice were seizure resistant.[14] Therefore, the role of LOF of *SCN8A* in the pathogenesis of epileptic encephalopathy remains to be confirmed.

Potassium channel genes

Potassium channels compose a large and diverse superfamily of ion channels. Voltage-gated potassium channels (K_v) regulate outward K^+ currents that contribute to membrane repolarization and hyperpolarization, thus limiting neuronal excitability.[15] Genes *KCNA1*, *KCNA2*, *KCNB1*, *KCNQ2*, and *KCNT1* have been reported to be associated with epileptic encephalopathy (Table 1, Supplementary Table S3 online).

Twelve missense variants in *KCNA2* were identified in 22 patients with epileptic encephalopathy (Table 1). R297, T374, and P405 residues appeared to be hotspots for variants. Functional studies on the Q213X, I263T, G398C, and P405L variants demonstrated a dramatic reduction of current amplitudes (complete LOF) and a dominant-negative effect on wildtype $K_v1.2$ channels. In contrast, the E157K, R297Q, and L298F variants induced a drastic GOF that caused permanent channel opening. Most patients with LOF variants had focal seizures that responded well to antiepileptic drugs and half of them became seizure-free.[16] Patients with GOF variants, however, presented generalized seizures and had less favorable outcomes with more severe intellectual disability,[16] suggesting a funotype–phenotype correlation. However, the detailed mechanism for GOF of the $K_v1.2$ channel that leads to epilepsy remains unclear.

Nine *KCNB1* missense and one truncating variants were reported in unrelated patients with epileptic encephalopathy (Table 1). Functional studies were performed in six variants and revealed a LOF effect,[17,18] explaining their roles in epileptic encephalopathy.

More than 80 variants in *KCNQ2* have been previously reported, mainly in patients with benign familial neonatal seizures.[19] Additionally, 66 de novo missense variants were identified in epileptic encephalopathy, using conventional or NGS methods,[20] with frequencies up to 19.0% (16/84) in Ohtahara syndrome (Table 1). A previous study demonstrated a reduction of 25% in the M-current amplitude was sufficient to increase neuronal excitability to epileptogenic levels and cause epilepsy.[21] The majority of variants associated with epileptic encephalopathy exhibited a LOF and dominant-negative effect.[22] A functional comparison between the R213W and R213Q variants, which were from benign familial neonatal seizures and neonatal epileptic encephalopathy, respectively, demonstrated that R213Q caused more severe functional impairment,[23] suggesting that *KCNQ2* phenotype severity was correlated with functional deficiency. Several variants associated with epileptic encephalopathy revealed a GOF effect by stabilizing the activated state of the channels,[24]

but their association with epileptic encephalopathy and the underlying mechanism warrant further study.

KCNT1 variants were first reported in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE).[25] De novo variants have also been frequently identified in epileptic encephalopathy, mostly in EIMFS (77.1%, 37/48) (Table 1). All known functional consequences of variants associated with EIMFS and ADNFLE displayed a strong GOF effect by producing large-fold increases in the current amplitude.[26–28] EIMFS-associated variants caused a significantly larger current than ADNFLE-associated variants.[28]

GABA receptor genes

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter of the CNS, exerts its action via two types of receptors, i.e., ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. Five genes encoding $GABA_A$ subunits, including *GABRA1*, *GABRB1*, *GABRB2*, *GABRB3*, and *GABRG2*, have been reported in epileptic encephalopathy (Supplementary Tables S4 online).

All known functional consequences of epileptic encephalopathy-associated $GABA_A$ mutants were LOF (Supplementary Table S8 online), which differed from the diverse functional alterations in sodium or potassium channels. *GABRA1*, *GABRB3*, and *GABRG2* have been previously reported in mild idiopathic generalized epilepsies, i.e., typically epilepsies characterized by absences.[29] Their associations with epilepsy were supported by experimental evidence, including studies on genetic knock-out or knock-in models.[30–32] Using NGS, a number of de novo variants in these genes have been identified in intractable epileptic encephalopathy. Recent studies have demonstrated that *GABRG2* variants associated with epileptic encephalopathy had a profound dominant negative effect, in contrast to pure haploinsufficiency in idiopathic generalized epilepsies, explaining the severe epileptic encephalopathy caused by *GABRG2* variants.[31] However, the factors that influence phenotype severity and their roles in epileptic encephalopathy are still elusive for other genes.

Glutamate receptor genes

N-methyl-D-aspartate receptors are predominant excitatory neurotransmitter receptors. Variants in *GRIN1*, *GRIN2A*, *GRIN2B*, and *GRIN2D* have been identified in epileptic encephalopathy (Table 1, Supplementary Table S4 online).

To date, 15 de novo heterozygous variants in *GRIN1* have been reported in 23 cases,[33,34] including 7 variants in epileptic encephalopathy (Table 1). Severe intellectual disability associated with profound global developmental delay is a predominant feature of the phenotype. Approximately 70% of these patients had epilepsy of varying severity. Functional studies revealed a LOF effect of all *GRIN1* variants.[34] Homozygous *GRIN1* variants were associated with a more severe phenotype[34], suggesting a quantity-dependent feature

More than 60 *GRIN2A* variants have been reported, mostly associated with the focal epilepsy spectrum.[35] The majority of

Table 2 Genotype, functional alteration, and phenotype of variants in non-ion channel genes in epileptic encephalopathies

Gene	Genotype (variant no.)	Funotype (variant no.)	EE phenotype (case no.)	Previously reported phenotype (inheritance)
Transporter/receptor				
ATP1A2	Missense (2)	UD (2)	uEE (2)	FHM[a] (AD)
NTRK2	Missense (2)	UD (2)	WS (3), uEE (2)	OBHD (de novo)
SLC2A1	Missense (1)	LOF (1)	uEE (1)	Dystonia[a] (AD)
	Truncating (1)	Destructive (1)	uEE (1)	/
SLC6A1	Missense (4)	UD (4)	EMAS (4)	/
	Truncating (2)	Destructive (2)	EMAS (2)	/
STXBP1	Missense (22)	LOF (1)	WS (1)	/
		UD (21)	OS (8), WS (5), DS (3), LGS (1), uEE (14)	uEE (de novo)
	Truncating (9), splice (6), GR (4)	Destructive (19)	OS (7), WS (2), uEE (11)	MR[a] (de novo)
Signaling molecule				
FGF12	Missense (1)	GOF (1)	uEE (7)	/
YWHAG	Missense (3)	UD (3)	LGS (1), uEE (4)	/
Cytoskeletal protein				
DYNC1H1	Missense (2)	UD (2)	WS (2)	MR (de novo or AD[a])
SPTAN1	Missense (1)	UD (1)	uEE (1)	/
	Truncating (2)	Destructive (2)	WS (1), uEE (1)	GE (de novo)
Nucleic acid binding protein				
ANKRD11	Truncating (2)	Destructive (2)	WS (1), uEE (1)	KBGS[a] (AD or de novo)
EEF1A2	Missense (3)	UD (3)	WS (2), LGS (1), uEE (5)	MR[a] (de novo)
FOXG1	Missense (1)	UD (1)	LGS (1), WS (1)	RTT[a] (unknown)
	Truncating (1)	Destructive (1)	uEE (1)	RTT[a] (de novo)
NACC1	Missense (1)	UD (1)	WS (4)	NDD[a] (de novo)
Enzyme				
CHD2	Missense (3)	UD (3)	uEE (3)	/
	Truncating (6), splice (2)	Destructive (8)	DS (2), EMAS (2), LGS (2), uEE (2)	/
DNM1	Missense (10)	pLOF (3)	LGS (2), WS (1)	/
		UD (7)	WS (5), LGS (1), uEE (2)	/
DNM1L	Missense (1)	LOF (1)	uEE (2)	EMPF[a] (de novo)
GNAO1	Missense (11)	pLOF (2), LOF (1)	OS (2), WS (1), uEE (5)	/
		UD (8)	WS (3), OS (1), uEE (5)	NEDIM[a] (de novo)
	Truncating (1)	Destructive (1)	OS (1)	/
HECW2	Missense (3)	UD (3)	WS (2), uEE (3)	NDD[a] (de novo)
NEDD4L	Missense (2)	UD (2)	WS (2)	PNH (de novo or AD[a])
Enzyme modulator				
SYNGAP1	Missense (1)	UD (1)	WS (1)	MR[a] (de novo)
	Truncating (5)	Destructive (5)	uEE (5)	MR (de novo[a] or AD)

Genes with pathogenic potential to be confirmed are not listed here. Details in Supplementary Table S 5 online
AD autosomal dominant, *DS* Dravet syndrome, *EE* epileptic encephalopathy, *EMAS* epilepsy with myoclonic–atonic seizures, *EMPF* encephalopathy due to defective mitochondrial and peroxisomal fission, *FHM* familial hemiplegic migraine, *GE* generalized epilepsy, *GOF* gain of function, *GR* genomic rearrangement, *KBGS* KBG syndrome, *LGS* Lennox–Gastaut syndrome, *LOF* loss of function, *MR* mental retardation, *NDD* neurodevelopmental disorder, *NEDIM* neurodevelopmental disorder with involuntary movements, *OBHD* obesity, hyperphagia, and developmental delay, *OS* Ohtahara syndrome, *pLOF* partial loss of function, *PNH* periventricular nodular heterotopia, *RTT* Rett syndrome, *UD* functional alteration not determined missense, *uEE* unspecified epileptic encephalopathy, *WS* West syndrome
[a]Phenotype with seizures

variants were found in families with incomplete penetrance or intrafamilial variability. Previous studies have observed a higher mutation frequency in the more severe phenotype.[36] Using NGS technologies, three *GRIN2A* variants were found in epileptic encephalopathy other than Landau–Kleffner syndrome and ECSWS (Table 1). Most functional studies on *GRIN2A* mutations demonstrated a GOF effect,[37] suggesting that *GRIN2A* variants with GOF may contribute to epileptic encephalopathy, particularly Landau–Kleffner syndrome and ECSWS, whereas the role of variants with other funotypes remains elusive.[38]

GRIN2B variants were initially identified in patients with intellectual disability or autism spectrum disorder.[39,40] Thirteen de novo variants were detected in patients with epileptic encephalopathy from different cohorts (Table 1). The missense variants N615I and V618G from West syndrome dramatically increased Ca^{2+} influx (GOF), while the R540H variant from focal epilepsy caused a milder GOF effect,[41] implying a potential quantitative correlation between the functional alteration and clinical severity.

Other ion channel genes

HCN1 is one of four genes encoding hyperpolarization-activated, cyclic nucleotide-gated channels (HCNs) with permeation to Na^+ and K^+ (I_h current). A study detected six missense variants in patients with early infantile epileptic encephalopathy characterized as fever-sensitive.[42] Electrophysiological studies showed either a LOF or GOF effect of the variants (Table 1).[42] The *Hcn1* knockout mice displayed increased excitability and sensitivity to convulsants,[43] supporting the association between *HCN1* LOF and epilepsy.

Non-ion channel genes

A growing number of non-ion channel genes are potentially associated with epileptic encephalopathy. All functionally determined variants in non-ion channel genes caused a LOF, except those in *FGF12*. Potential epileptic encephalopathy–associated genes included *CHD2*, *DNM1*, *FGF12*, *GNAO1*, *SLC6A1*, *SPTAN1*, and *STXBP1* (Table 2, Supplementary Table S5 online).

The *CHD2*-encoded CHD2 protein is a member of the chromodomain helicase DNA-binding proteins and acts to modulate chromatin structure.[44] Eleven *CHD2* de novo variants have been identified in patients with epileptic encephalopathy such as Lennox–Gastaut syndrome and EMAS. The majority of variants were destructive, suggesting a role of haploinsufficiency in pathogenesis. An in vivo zebrafish model system revealed that *chd2*-knockdown larvae exhibited an abnormal movement pattern with epileptiform discharges.[45]

DNM1 encodes dynamin-1, a brain-expressed GTPase, which is involved in activity-dependent synaptic vesicle recycling.[46] Ten de novo missense variants have been identified in epileptic encephalopathy. Functional studies on A177P, K206N, and G359A revealed the impaired endocytosis of transferrin, in a dominant-negative manner.[47] A mouse model with heterozygous A408T missense variant developed recurrent limbic and generalized tonic–clonic seizures, while homozygous mice exhibited a more severe phenotype.[48]

FGF12 (also known as *FHF1*) encodes a small cytosolic protein that interacts with the cytoplasmic tails of voltage-gated sodium channels. *FGF12* can promote excitability by delaying fast inactivation of the channels. A recurrent de novo *FGF12* variant (R52H) was reported recently in seven unrelated patients with epileptic encephalopathy (Table 2).[49] All affected individuals presented with early-onset intractable seizures, mostly tonic seizures and frequent status epilepticus. Functional studies showed enhanced modulation of channel inactivation gating, equivalent to a GOF effect.[49] The *fhf1b1* R56H (corresponds to R52H in h*FGF12*) knock-in zebrafish model displayed recurrent epileptiform discharges in 50% of larvae, indicating significantly higher epileptic potential than controls.[49]

GNAO1 encodes $G\alpha_o$, which is the α-subunit of heterotrimeric guanine nucleotide-binding proteins (G-proteins). De novo heterozygous *GNAO1* variants have been identified in Ohtahara syndrome, West syndrome, and other forms of epileptic encephalopathy (Table 2). Transient-expression studies demonstrated that the D174G, T191_F197del, and I279N variants impaired normal protein localization to the plasma membrane. Electrophysiological analysis demonstrated that D174G, T191_F197del, and G203R variants resulted in decreased $G\alpha_o$-mediated inhibition of calcium currents by norepinephrine.[50] Furthermore, a *Gnao1* G184S knock-in mouse model displayed seizures and an increased frequency of interictal epileptiform discharges on EEG.[51]

SLC6A1 encodes a voltage-dependent GABA transporter (GAT-1); its expression reflects GABAergic pathways.[52] Six *SLC6A1* variants were identified in seven cases with EMAS from a cohort of 644 patients with epileptic encephalopathy. They accounted for 3.8% of the probands (6/160) with EMAS (Supplementary Table S5 online). Five of them were de novo variants, among which one was passed on to the next generation with the same phenotype. Another variant was inherited from an unaffected mother of mosaicism,[53] indicating a potential quantity-dependent feature. It is therefore possible that *SLC6A1* variants are specifically associated with EMAS.

SPTAN1 encodes α-II spectrin, a filamentous cytoskeletal protein that regulates the stability of axonal structure. To date, eight de novo variants have been identified, including three identified by NGS (Supplementary Table S5 online). A hotspot variant, D2303_L2305 dup, has been identified in three patients with West syndrome. The phenotypes of *SPTAN1* variants included epileptic encephalopathy and generalized epilepsy with intellectual disability.[54] Experimental studies showed that variants with deletion or duplication in the last two spectrin repeats could lead to pathological aggregation of α/β spectrin heterodimer, and subsequently, the disturbed clustering of ankyrin G and voltage-gated sodium channels at axon initial segment, together with an elevated action potential threshold.[55] An in-frame deletion variant of *SPTAN1* showed less severe consequence of abnormal aggregation and was associated with relatively mild generalized epilepsy with intellectual disability,[54] suggesting a potential quantitative relationship.

The association between *STXBP1* de novo variants and epileptic encephalopathy has been demonstrated in previous studie[56,57] Recently, more variants have been identified in epileptic encephalopathy, further supporting the role of *STXBP1* variants in epileptic encephalopathy.

X-linked genes

X-linked genes are discussed separately because their variants affect male and female patients differently. Because males have one copy of the X chromosome, while females have two copies, a pathogenic heterozygous variant (hemizygous) in the X chromosome causes relatively more severe genetic damage in males. Thus, male patients with X-linked gene variants are more severely affected than female patients. Such a quantity-dependent feature is an additional consideration in the assessment of pathogenicity of de novo variants.

All X-linked genes in epileptic encephalopathy have previously been reported to be associated with epilepsy or seizures (Supplementary Tables S6, S9 online). Current data from functional studies and the common destructive variants suggest a role of LOF or haploinsufficiency of these genes in the epileptogenesis of epileptic encephalopathy (Supplementary Table S9 online). Differences in phenotype severity between male and female patients were observed in *CASK*, *CDKL5*, *CLCN4*, *IQSEC2*, *SLC35A2*, and *WDR45* (Supplementary Table S10 online).

CASK, *DCX*, and *PCDH19* are reportedly associated with epileptic encephalopathy previously.[58–61] Using NGS techniques, novel de novo variants in these genes have been identified in patients with epileptic encephalopathy (Supplementary Table S6, S9 online), suggesting a role of these variants in epileptic encephalopathy. *PCDH19* gene shows an unusual X-linked inheritance with highly selective involvement of female patients, except for several male patients with somatic mosaicism.[62] *PCDH19* encodes protocadherin 19, which plays roles in neuronal migration and synaptic plasticity. Most variants arose de novo in the sporadic cases, with a few being transmitted from asymptomatic parents or affected mothers.[63] Hemizygous males are clinically unaffected. This special phenomenon could be attributed to a mechanism known as "cellular interference,"[61] i.e., a heterogeneous population of mutant and wildtype cells are required for disease to occur; while a homogeneous cell population (either mutant or wildtype) does not lead to disease.

CDKL5/STK9 encodes a serine/threonine kinase protein with a high expression level in mature neurons in the cerebral cortex and hippocampus. *CDKL5* variants have been reported in diverse phenotypes, including Rett syndrome, epileptic encephalopathy, and autism.[58,64] In previous studies, the majority of *CDKL5* variants have been identified in girls, with only a few being reported in boys. *CDKL5* variants were found in an estimated 8–16% of girls with early onset seizures, and more specifically in 28% of girls with early onset seizures combined with West syndrome.[64] However, affected boys showed a more severe clinical manifestation.[64] Using NGS, an additional 51 de novo variants were identified in severe epileptic encephalopathy cases, which included quite a few boys (Supplementary Table S6 online).

CLCN4 encodes the voltage-dependent $2Cl^-/H^+$-exchanger ClC-4. *CLCN4* variants have been previously identified in families with X-linked intellectual disability and seizures,[65,66] and affected male patients had a more severe phenotype than

female patients.[66] The identification of two de novo variants in boys with severe epileptic encephalopathy further supported the pathogenic role of *CLCN4* (Supplementary Table S6 online).

IQSEC2 encodes a guanine nucleotide exchange factor for the ADP ribosylation factors family of small GTP-binding proteins. Its variants have been previously implicated in X-linked intellectual disability. Moreover, epilepsy is a common finding in the patients.[67] Boys usually have a more severe phenotype than girls.[67] Ten de novo *IQSEC2* variants were recently identified in patients with epileptic encephalopathy, including four variants in male patients (Supplementary Tables S6 online).

SLC35A2 encodes a Golgi-localized UDP-galactose transporter, which imports UDP-galactose from the cytoplasm to the lumen of the Golgi apparatus. *SLC35A2* was reported previously in four patients with congenital disorder of glycosylation, among whom three showed seizures and hypsarrhythmia on EEGs.[68,69] Recently, nine de novo *SLC35A2* variants were identified in patients with epileptic encephalopathy (Supplementary Table S6 online), accompanied by characteristic hypsarrhythmia on EEGs.[69,70]

WDR45, which encodes a WD40-repeat protein that plays an important role in the autophagy pathway, is associated with a subtype of neurodegeneration with brain iron accumulation.[71] Six novo variants have been reported in patients with epileptic encephalopathy (Supplementary Table S6 online), among whom boys were more severely affected.[72,73]

EVIDENCE IMPLICATING THE PATHOGENIC POTENTIAL OF GENES

In analyzing the associations between epileptic encephalopathy and the genes, evidence from several aspects indicated a general association between epilepsy and the genes, which were considered to support a possibly pathogenic role of the genes in epileptic encephalopathy. These include the following: (1) significantly high frequency or hotspot of variants in the patient group, (2) other epilepsy-related phenotype with defined inheritance pattern, (3) variants in the gene result in functional impairment that is related to epilepsy. Genes with at least one of these characteristics (Table 3) were considered possibly pathogenic.

Generally, a significantly higher frequency of variant helps determine the association between a gene and a genetic disease.[3] De novo variants were identified in rare cases of each independent epileptic encephalopathy cohort, which varied considerably in size (Supplementary Tables S2–7 online). Therefore, except the de novo *SCN1A* variants in Dravet syndrome cases,[6] it was difficult to determine the frequency of de novo variants in most epileptic encephalopathies. However, hotspot variants were found in several genes (Table 3).

Familial epilepsies with dominant inheritance have been reported previously in several genes (Table 3), supporting their possible roles in epileptic encephalopathy. Theoretically, although a heterozygous de novo variant may compose a

homozygous (or compound heterozygous) variant with an inherited variant and present a recessive inheritance pattern,[74] this has not been observed in epileptic encephalopathy.

Functional studies on variants have been performed for most ion-channel genes and several non-ion channel genes. The functional impairments explained epileptogenesis in the majority of the epileptic encephalopathy-related genes, such as LOF of GABAergic receptors. However, the funotype of variants in several genes could not explain the occurrence of epilepsy, such as the LOF of the *SCN8A* gene.[14]

Evidence with several characteristics was found to be specific in supporting the pathogenic role of genes in epileptic encephalopathy. This included the following: (1) variants of the gene are associated with characteristic phenotype; (2) distinct correlation between genotype/funotype and phenotype; (3) correlation between genetic impairment and phenotype severity, explaining the severe phenotype of epileptic encephalopathy; and (4) variant-specific model with corresponding phenotype or defined mechanisms of epileptogenesis. Supplementary Table S10 online summarizes the evidence for such genes with de novo variants, which were recurrently identified in unrelated cases.

First, two genes are associated with characteristic epileptic encephalopathy phenotypes. *SLC6A1* mutations were identified in EMAS cases alone, but not in cases with other forms of epileptic encephalopathy.[53] *SLC35A2* variants were associated with West syndrome with hypsarrhythmia on EEGs.[69,70]

Second, variations of several ion channel genes elicited diverse functional alterations, including those in *GRIN2A*, *GRIN2B*, *HCN1*, *KCNA2*, *KCNQ2*, *SCN1A*, *SCN2A*, and *SCN8A*. Distinct funotype–phenotype correlation could be found in *SCN1A* and *KCNA2*. All epileptic encephalopathy-related *SCN1A* variants were featured by LOF, whereas GOF variants were associated with mild generalized epilepsy with febrile seizures plus.[6] Similarly, *KCNA2* LOF variants were associated with partial epilepsy that responded well to antiepileptic treatment, in contrast to GOF variants that tended to cause generalized epilepsy with less favorable outcomes.[16]

Third, a correlation between genetic impairment and phenotype severity was found in several genes, explaining the severity of epileptic encephalopathy (Supplementary Table S10 online).

Fourth, variant-specific knock-in models have produced epileptic encephalopathy for several genes, or epileptogenesis mechanism has been defined, providing direct evidence of their causative role in epileptic encephalopathy (Supplementary Table S10 online).

Based on the analysis of evidence supporting pathogenicity, we summarized the pathogenic potential of epileptic encephalopathy–associated genes and their pathogenic genotype and funotype (Table 4). A pathogenic or possible pathogenic role with defined funotype could be validated in 51 genes, whereas the pathogenic role of 23 genes warrants

Table 3 Summary of evidence implicating possibly pathogenic genes in epileptic encephalopathies

Gene	Hotspot variant (no.)	Other dominantly inherited epilepsy phenotype	Funotype related to epilepsy
Ion channel			
SCN2A	A263 (4), R853 (4),	BFNS	GOF
	E999 (3), L1342 (3),	BFNS	LOF?
	R1882 (5)		
SCN8A	R850 (3), R1617 (4),	BFIS (UD)	GOF
	A1650 (3), R1872		LOF?
	(13)		
KCNA1	/	EA/myokymia[a] (UD)	LOF?
KCNA2	R297 (7), T374 (3),	HSP[a]	LOF
	P405 (4)	/	GOF?
CACNA1A	A713 (3)	EA/FHM[a]	/
GABRA1	M263 (3)	IGE, JME	LOF
GABRB1	/	/	LOF
GABRB2	Y244 (4)	/	LOF
GABRB3	/	CAE	LOF
GRIN2A	/	FE	GOF
		FE	LOF?
GRIN2D	/	/	GOF
HCN1	/	/	LOF
	/	/	GOF?
Non-ion channel			
ANKRD11	/	KBGS[a]	/
ATP1A2	/	FHM[a]	/
DNM1L	/	/	LOF
DYNC1H1	/	MR[a]	/
EEF1A2	G70 (4), E122 (3)	/	/
FOXG1	/	RTT[a]	/
HECW2	R1330 (3)	/	/
NACC1	R298 (4)	/	/
NEDD4L	/	PNH[a] (UD)	/
NTRK2	Y434 (4)	/	/
SLC2A1	/	Dystonia[a]	LOF
SYNGAP1	/	/	LOF
YWHAG	R132 (3)	/	/
X-linked			
ALG13	N107 (6)	/	/
MECP2	/	RTT[a]	LOF
PIGA	/	/	LOF
SLC9A6	/	MR[a]	/

Hotspot variants listed here are those that have been observed in no less than three unrelated cases

BFIS benign familial infantile seizures, *BFNS* benign familial infantile seizures, *CAE* childhood absence epilepsy, *EA* episodic ataxia, *FE* focal epilepsy, *FHM* familial hemiplegic migraine, *GOF* gain of function, *HSP* hereditary spastic paraplegia, *IGE* idiopathic generalized epilepsies, *JME* juvenile myoclonic epilepsy, *KBGS* KBG syndrome, *LOF* loss of function, *MR* mental retardation, *PNH* periventricular nodular heterotopia, *RTT* Rett syndrome, *UD* functional alteration not determined missense

[a]Phenotype with seizures

Table 4 Summary of pathogenic potential, functional alteration, and genotype of genes in epileptic encephalopathies

	Pathogenic	Possibly pathogenic	To be confirmed
GOF (missense)	***Ion channel***		
	KCNA2, KCNT1; GRIN2A, GRIN2B	SCN2A, SCN8A; GRIN2D	KCNQ2; HCN1
	Non-ion channel		
	FGF12		
LOF or destructive	***Ion channel***		
	SCN1A; KCNA2[a], KCNB1, KCNQ2; GABRG2[a]; GRIN1[a]	SCN2A; GABRA1, GABRB1[a], GABRB2[a], GABRB3; GRIN2A[a]; HCN1[a]	SCN8A; GRIN2B
	Non-ion channel		
	CHD2, DNM1[a], GNAO1, SLC6A1, SPTAN1, STXBP1	ANKRD11, DNM1L[a], SLC2A1, SYNGAP1	DYRK1A, HNRNPU, KMT2A, NUS1, TRIM8, ZEB2
	X-linked		
	CASK, CDKL5, CLCN4[a], DCX, IQSEC2, PCDH19[b], SLC35A2, WDR45	MECP2, SLC9A6	SMC1A
UD		ALG13, ATP1A2, CACNA1A, DYNC1H1, EEF1A2, FOXG1, HECW2, KCNA1, NACC1, NEDD4L, NTRK2, PIGA, YWHAG	BCL11A, CLTC, COL4A1, DHDDS, FASN, GABBR2, MEF2C, NAA10, NR2F1, PIK3AP1, RANGAP1, RYR3, SIK1, SLC1A2, SNAP25, TBL1XR1

Underline, gene with diverse functional alterations

GOF gain of function, *LOF* loss of function, *UD* functional alteration not determined missense

[a]Genes with variants of only missense that revealed a functional alteration of LOF

[b]Special mechanism other than LOF and GOF

Table 5 Guideline for evaluation of pathogenic potential of genes with de novo variants in epileptic encephalopathies

Genes with de novo variants recurrently identified in unrelated cases are subject to further evaluation of pathogenic potential.

Possibly pathogenic with at least one of the following evidence:
1. Significantly high frequency or hotspot of variants in the patient group.
2. Other epilepsy-related phenotype with defined inheritance pattern.
3. Variants in the gene result in functional impairment that is related to epilepsy.
Pathogenic with at least one of the following specific evidence:
1. Variants of the gene are associated with characteristic phenotype.
2. Distinct correlation between genotype/funotype and phenotype.
3. Correlation between genetic impairment and phenotype severity, and it explains the severe phenotype.
4. Variant-specific model with corresponding phenotype, or defined mechanisms of epileptogenesis.

further validation. Variants of diverse functional alterations were found in several genes; and in four of these genes, the pathogenicity of one of the funotypes could not be determined. Table 5 summarizes the evidence implicating the pathogenic potential of genes associated with epileptic encephalopathies.

SUMMARY

The evaluation of de novo heterozygous variants identified in patients with epileptic encephalopathy is a major challenge. Current guidelines have provided useful, but relatively general, tools for evaluating the causality of these variants.[3,4,75] However, epileptic encephalopathies are specific age-related conditions with heterogeneous clinical phenotypes, multiple etiologies, and complex mechanisms of epileptogenesis. Therefore, the evaluation of the pathogenic potential of epileptic encephalopathy-related genes, i.e., the pathogenicity at gene level, is critical. The present data showed that previously reported epileptic encephalopathy-related genes differ widely in their association with epileptic encephalopathy. Based on currently available data, 24 genes were estimated to be pathogenic and 27 genes were estimated to be possibly pathogenic in epileptic encephalopathies. Furthermore, epileptic encephalopathy-related genes had specific pathogenic funotypes, such as GOF *KCNT1* variants and LOF *GABRG2* variants. Even genes with a similar function and structure or ones that involved in the same functional pathway, such as sodium channel genes *SCN1A*, *SCN2A*, and *SCN8A*, however, differ in their pathogenic potential in epileptic encephalopathies, including their pathogenic genotypes and funotypes. A total of 23 genes require further study to determine their pathogenic role or pathogenic funotype in epileptic encephalopathy. Thus, the pathogenicity of variants in these genes should be considered conservatively. The data suggest that evaluating the pathogenic potential of genes is required before considering the pathogenicity of variants in a clinical or genetic consulting

practice. The summary of epileptic encephalopathy-associated genes and their pathogenic genotype and funotype (Table 4) may be used to help evaluate the pathogenicity of variants identified in an individual with epileptic encephalopathy.

Based on evidence in supporting pathogenicity, we outlined a guideline for evaluating the pathogenic potential of genes associated with epileptic encephalopathy (Table 5). From a clinical genetics point of view, characteristic phenotype, distinct genotype/funotype–phenotype correlation, and correlation between genetic impairment and phenotype severity appeared to be specific considerations when evaluating the pathogenic potential of genes related to epileptic encephalopathy. The summarized data is expected to be helpful in outlining guidelines for evaluating the pathogenic potential of genes associated with other genetic diseases.

ELECTRONIC SUPPLEMENTARY MATERIAL

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DISCLOSURE

The authors declare no conflicts of interest.

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