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REVIEW

Mutations of *N*-Methyl-D-Aspartate Receptor Subunits in Epilepsy

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Abstract Epilepsy is one of the most common neurological diseases. Of all cases, 70%–80% are considered to be due to genetic factors. In recent years, a large number of genes have been identified as being involved in epilepsy. Among them, *N*-methyl-D-aspartate receptor (NMDAR) subunit-encoding genes represent a large proportion, suggesting an important role for NMDARs in epilepsy. In this review, we summarize and analyze the genotypes, functional alterations, and clinical aspects of NMDAR subunit mutations/variants identified from patients with epilepsy. These data will help to throw light upon the pathogenicity of these NMDAR mutations and advance our understanding of the subtle and complicated role of NMDARs in epilepsy. It will also offer new insights into precision therapy for this disorder.

Keywords Epilepsy · NMDA receptors · Subunit · Mutation

Introduction

Epilepsy is one of the most common neurological conditions, characterized by abrupt, recurrent, and synchronous discharges of the brain. Seventy to eighty percent of epilepsy cases are believed to be due to one or more genetic factors [1]. Advances in genomic technology have led to a rapid increase in the discovery of novel epilepsy-associated genes. Among these, a large proportion comprises ion channels and neurotransmitter receptors [2–4]. In particular, a surprising number of *N*-methyl-D-aspartate receptor (NMDAR) subunit mutations have been found in seizure disorders causing various childhood epilepsy syndromes, suggesting that the NMDAR subunit appears to be a locus for epilepsy [2, 3, 5].

NMDARs are a subtype of ionotropic glutamate receptors, mainly localized at the postsynaptic neuronal membrane. Unlike other kinds of such receptors, NMDARs possess some unique features including co-agonist activation, voltage-dependent blockade by extracellular Mg^{2+}, high permeability to Ca^{2+}, and relatively slow gating and deactivation kinetics [6–9]. There are at least seven NMDAR subunits, namely, GluN1, GluN2A-2D, and Glu3A-3B. The canonical NMDARs are heterotetrameric complexes usually composed of two glycine/D-serine-binding obligatory GluN1 subunits and two glutamate-binding regulatory GluN2 subunits (NR2A-2D). The GluN1/GluN2A/GluN2B triheteromer is the dominant NMDAR subtype that is widely distributed in the hippocampus and cortex [10–12]. Recently, the structures of the GluN1/GluN2B diheteromer and the GluN1/GluN2A/GluN2B triheteromer have been resolved by cryogenic electron microscopy. The mechanisms of NMDAR activation/inhibition and their allosteric modulation have been clarified by these studies, leading to further understanding of NMDARs [13, 14]. Typically, the receptor subunit possesses four discrete modules [6, 15]. The extracellular amino-terminal domain (ATD) is mainly involved in subunit oligomerization/assembly and allosteric regulation. The ligand-binding domain (LBD) is made up of two discontinuous segments (S1 + S2). The transmembrane domain (TMD) includes three and a half transmembrane

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helices (TM1-4) and forms the channel pore. The intracellular carboxyl-terminal domain (CTD) is highly flexible and is associated with receptor trafficking, anchoring, and signaling *via* interaction with various postsynaptic proteins [16–21].

Profile of NMDAR Subunit Mutations in Human Epilepsy

The *GRIN1* gene located at human chromosome 9q34.3 encodes the NMDAR GluN1 subunit. So far, twelve *GRIN1* mutations, including one duplication mutation, one nonsense mutation, and ten missense mutations, have been identified in epilepsy by targeted panel sequencing or whole-exome sequencing (Table 1). Of these twelve, eight have been functionally tested to show that six are loss-of-function mutations (D552E, Q556*, S560dup, Y647S, G815R, and G827R) and two are mutations without any functional change (A645S and R844C) [22, 23] (Fig. 1; Tables 1, 4). The S688Y mutation is considered to be located at the glycine-binding site. *In silico* studies have suggested that this mutation would disrupt NMDAR ligand binding, although functional investigation is lacking [24]. These twelve mutations are all *de novo* mutations except for Q556*. The Q556* truncation mutation was identified from three siblings with severe neonatal epileptic encephalopathy and is the only homozygous mutation. All three siblings died soon after birth, suggesting a critical function of the *GRIN1* gene during neurodevelopment. Note that D552E, Q556*, G815R, G827R, and R844C have been identified from two or more cases, indicating a greater likelihood of their pathogenicity in epilepsy. So far, no gain-of-function mutations have been found in the *GRIN1* gene from epilepsy cases.

The *GRIN2A* gene maps to human chromosome 16p13.2 and encodes the NMDAR GluN2A subunit. 16p13.2 is located at the classical 16p13.3p13.13 hotspot, which is one of the less stable regions of the human genome [28]. Since the first GluN2A mutation N615K was discovered in epilepsy in 2010 [29], eighty-two *GRIN2A* gene mutations have been identified in succession, including deletions, duplications, splice-site variations, nonsense mutations, and missense mutations (Table 2). The majority are missense mutations. The number of *GRIN2A* mutations is far greater than that of other NMDAR subunit mutations, suggesting that the *GRIN2A* gene is one of the most closely-related epilepsy genes. Thirty-two of the *GRIN2A* mutations have been investigated and twenty-eight of them have been shown to have functional alterations. Among these functionally validated *GRIN2A* mutations, nine are gain-of-function, nineteen are loss-of-function, and the remaining four have been found not to change NMDAR function (Fig.1; Tables 2, 4). So far, no homozygous mutations in *GRIN2A* have been reported, and the number of inherited mutations is far greater than those that are *de novo*. Both the inherited missense mutation V967L and the splice-site variant F139fs (predicted) have been found in twelve cases, indicating a likely role for these two mutations in epilepsy. M817V is the only *de novo* mutation discovered in two sporadic cases. The D731N mutation has been identified in two sporadic cases (*de novo*) as well as in a family (inherited).

The *GRIN2B* gene is located on human chromosome 12p13.1 and encodes the NMDAR GluN2B subunit. At the present time, only thirteen *GRIN2B* gene mutations have been identified as being associated with epilepsy, including two deletions, one inversion, one splice-site variant, and nine missense mutations (Table 3). Five missense mutations among them have been functionally analyzed. Three of these lead to gain-of-function effects of NMDARs (R540H, N615I, and V618G), while the other two have loss-of-function effects on the receptor (C436R and C461F) (Fig. 1; Tables 3, 4). Unlike epilepsy-associated *GRIN2A* mutations, the main form of *GRIN2B* mutations is *de novo* mutation, similar to *GRIN1*. To date, no epilepsy-associated missense mutation has been discovered in the C-terminus of the GluN2B subunit. This may suggest that the GluN2B C-terminus is more evolutionarily conserved and thus plays a key role in receptor trafficking and downstream signaling [6]. Unlike the epilepsy-associated *GRIN1* and *GRIN2A* mutations, no single mutation in *GRIN2B* has been discovered in more than one case.

The *GRIN2D* gene located on human chromosome 19 (19q13.3) encodes the NMDAR GluN2D subunit. Only one *GRIN2D* mutation has been identified in epilepsy (Table 4). It is the missense, heterozygous, *de novo* mutation V667I. This mutation located in GluN2D TM3 was identified in two unrelated children with epileptic encephalopathy. Functional analysis has revealed that this is a gain-of-function mutation [63].

According to the epilepsy-associated mutations of *GRIN* subunit genes identified to date, *GRIN2C, GRIN2D, GRIN3A*, and *GRIN3B* mutations seem to be more relevant to human intellectual disorders (IDs), autism, and schizophrenia [3, 43].

Relationship Between Mutation and Phenotype

It seems hard to predict the phenotype from any particular epilepsy-associated NMDAR mutation (Tables 1, 3). One reason is that genetic mutation is not the only factor resulting in epilepsy. Genetic background and environmental factors can also contribute to the phenotype.

In general, epilepsy-associated GluN1, GluN2B, and GluN2D mutations display more severe clinical phenotypes than GluN2A mutations and appear to be more susceptible

Table 1 Summary of *GRIN1* mutations identified in epilepsy

GRIN1	Protein (cases)	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
Duplication (1)	p.Ser560dup	c.1679_1681dupGCA	Het	*De novo*	S1-M1 linker	Receptor activity↓	LOF	Partial complex epilepsy + Severe ID + CVI	[22, 25]
Nonsense (1)	p.Gln556* (3)	c.1666C>T	Homo	Inherited	S1-M1 linker	Nonfunctional	LOF	Fatal EE (3)	[22]
Missense (10)	p.Ser549Arg	c.1654A>C	Het	*De novo*	S1-M1 linker			Epilepsy + Severe ID + MD	[22, 23]
	p.Asp552Glu (2)	c.1656C>A	Het	*De novo*	S1-M1 linker	Current↓, Glu↓, Gly↓	LOF	GTCS + Severe ID	[23, 26]
		c.1656C>A	Het	*De novo*				Epilepsy + Severe ID + MD + CVI	[22]
	p.Met641Ile	c.1923G>A	Het	*De novo*	M3			Epilepsy + Severe ID + MD	[22, 23]
	p.Ala645Ser	c.1933G>T	Het	*De novo*	M3	→	No change	Epilepsy + Severe ID + CVI	[22, 23]
	p.Tyr647Ser	c.1940A>C	Het	*De novo*	M3	Maximal agonist-inducible currents↓	LOF	IS + Severe ID	[22, 27]
	p.Asn650Lys	c.1950C>G	Het	*De novo*	M3			Epilepsy + Severe ID + MD	[22, 23]
	p.Ser688Tyr	c.2063C > A	Het	*De novo*	LBD (S2)			EOEE + Hyperkinetic and oculogyric-like movements	[24]
	p.Gly815Arg (4)	c.2443G>A	Het	*De novo*	M4	Maximal agonist-inducible currents↓, Glu↓	LOF	Epilepsy + Severe ID + MD	[23]
		c.2443G>A	Het	*De novo*				Epilepsy + Severe ID + MD + CVI (2)	[22]
		c.2444G>T	Het	*De novo*				Epilepsy + Severe ID + MD	[22]
	p.Gly827Arg (4)	c.2479G>A	Het		M4	Nonfunctional	LOF	Epilepsy + Severe ID + MD	[22]
		c.2479G>A	Het	*De novo*				Epilepsy + Severe ID + MD	[22]
		c.2479G>A	Het	*De novo*				Severe ID + MD	[22]
		c.2479G > A	Het	*De novo*				EOEE+ Hyperkinetic and oculogyric-like movements	[24]
	p.Arg844Cys (2)	c.2530C>T	Het	*De novo*	CTD	→	No change	Epilepsy + Severe ID + MD (2)	[22]

CTD C-terminal domain, *CVI* cortical visual impairment, *EE* epileptic encephalopathy, *EOEE* early-onset epileptic encephalopathy, *Het* heterozygous, *Hom* homozygous, *ID* intellectual disability, *LBD* ligand-binding domain, *LOF* loss-of-function, *M1-4* transmembrane domain 1-4, *MD* movement disorder, *S1-2* S1 and S2 segment of ligand binding domains

↓ Decrease, → no change

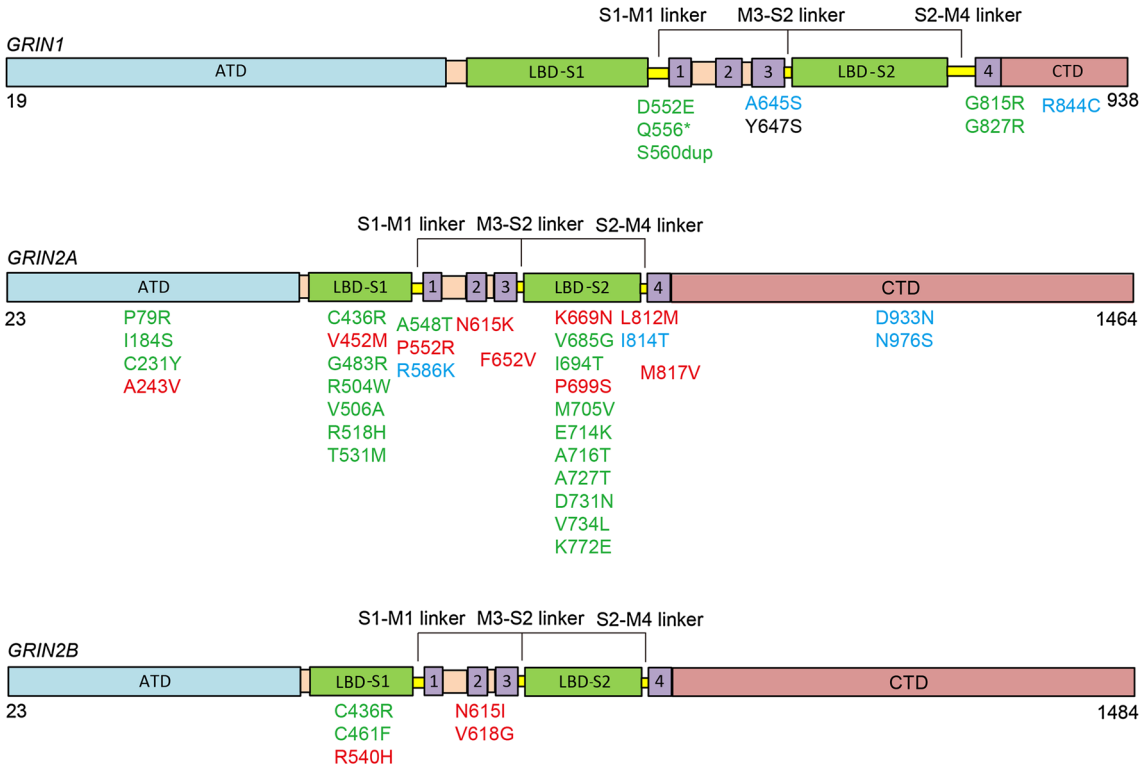

Fig. 1 Distribution of functionally evaluated *GRIN1, GRIN2A*, and *GRIN2B* mutations/variants associated with epilepsy. ATD, amino-terminal domain (blue); LBD-S1 and LBD-S2, the first and second polypeptide sequences comprising the LBD (green); 1, 2, 3, and 4, transmembrane domains (purple); CTD, carboxyl-terminal domain (pink). Linker regions (S1-M1 linker, M3-S2 linker, and S2-M4 linker) are in yellow. Mutations with gain-of-function effects, loss-of-function effects, and unchanged effects are indicated in red, green, and blue, respectively

to ID and developmental delay (DD), whereas GluN2A mutations are predominantly associated with language disorders [64]. GluN1, GluN2B, and GluN2D subunits are all expressed from the embryonic period, and their function cannot be replaced by the postnatally-expressed GluN2A [65–68], which may explain the more severe symptoms caused by these subunits. Furthermore, the GluN1 subunit is the obligatory subunit of the NMDAR channel widely expressed in the brain [69–71], which means that truncation of the two *GRIN1* alleles or missense mutation of GluN1 would result in more severe phenotypes in patients with epilepsy than GluN2 subunits. The etiology of the motor and language disorders caused by GluN2A mutations is elusive. GluN2A-containing NMDARs are extensively expressed in various brain regions, including motor speech areas. Dysfunction of NMDARs caused by *GRIN2A* mutations may disrupt speech and language networks, which may partially explain the motor and language disorders.

Patients with epilepsy carrying different mutations usually have different epileptic phenotypes (Tables 1–3). The epileptic phenotypes of patients with *GRIN2A* mutations cover a wide range. The common seizure types are benign epilepsy with centro-temporal spikes (BECTS), atypical benign partial epilepsy, continuous spike and slow-wave during sleep (CSWS), and Landau-Kleffner syndrome (LKS), and some of the patients display motor and language disorders [30, 33, 46] (Table 2). More severe phenotypes such as early-onset epileptic encephalopathy (EOEE) accompanied by DD or ID have also been reported [29, 72], indicating that the GluN2A-containing NMDARs also play an important role during neural development.

It is also not surprising that different mutations can lead to the same/similar clinical phenotypes (Tables 1–3). Epilepsy-associated *GRIN1* mutations show uniformly severe phenotypes [22] (Table 1). The genotype lacks correlation with the phenotype. A shared secondary mechanism led by changes in NMDAR subunit composition or trafficking is one of the possible explanations.

In most cases, the phenotypes of patients with the same mutation are identical, but this is not always true. Phenotypic heterogeneity exists in both *de novo* and inherited mutations (Table 2). GluN2A D731N was identified in three unrelated patients with epilepsy. The phenotypes of these patients are unexplained epilepsy accompanied by DD [51], LKS [52], and Rolandic epilepsy with language dysfunction [33]. For a few inherited

Table 2 Summary of *GRIN2A* mutations identified in epilepsy

GRIN2A	Protein (cases)	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
Deletion (16)	Not known	del chr16: 9 850 000–9 900 000 (hg19)	Het					ABPE	[30]
	Not known	del chr16: 9 825 000–10 075 000 (hg19)	Het					RE + Mild ID	[30]
	Not known	del chr16: 10 250 000–10 275 000 (hg19)	Het					RE	[30]
	Not known	del chr16: 7 964 000–10 607 500 (hg19)						Pseudo-Lennox Syndrome + Severe ID	[31]
	p.Lys592fs (predicted)	del chr16: 8 992 500–9 992 500 (hg19)			M2			RE + Moderate ID	[31]
	Not known	del chr16: 9 365 500–11 273 700 (hg19)						Myoclonic seizures + Severe ID	[31]
	p.Arg865fs (predicted)	del chr16: 9 809 522–9 856 618 (hg19)			CTD			RE + Mild ID	[32]
	p.Phe670fs (4)	del chr16: 9 908 477–9 934 830 (hg19) (2)		Inherited	LBD (S2)			LKS (proband); LKS + VD (two brothers); BCE	[33]
	Not known (3)	del chr16: 10 227 121–10 354 862 (hg19)		Inherited				CSWSS + VD (three brothers)	[33]
	Not known	16p13.2 microdeletion		Inherited				Focal seizures + Delayed cognition	[34]
	Not known	16p13.2p13.13 microduplication		*De novo*				Epilepsy + ID + Delayed speech	[35]
	p.Pro31Serfs*107 (3)	c.90delTins(T)2	Het	Inherited	ATD			RE (proband, brother, father)	[30]
	p.Phe528Glyfs*22	c.1586delT	Het		LBD (S1)			CSWS + Severe LD	[36]
	p.Val529Trpfs*22 (3)	c.1585delG	Het	Inherited	LBD (S1)			BECTS + Mild ID (proband, brother, father)	[30]
	p.Ser547del	c.1637_1639delCTT	Het		S1-M1 linker			ABPE/CSWS + ID	[30]
	p.Leu779Serfs*5	c.2334_2338delCTTGC	Het	Inherited	LBD (S2)			ABPE/CSWS	[30]

Table 2 continued

GRIN2A	Protein (cases)	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
Duplication (3)	Not known	dup chr16: 10 075 000–10 225 000 (hg19)	Het					CSWSS + ID	[30]
	Not known	c.2008-32_c.2008-31dupCT	Het					Epilepsy	[29]
	Not known	Exon 4 & 5	Het					EE	[36]
Translocation (1)	Not known (4)	t (16;17) (p13;q11)		Inherited				FS + GTCS + Severe ID (proband); GTCS + learning difficulties (father, aunt); Seizures + severe ID (cousin)	[29]
Splice-site (4)	Not known	c.414+7C>T		Inherited				IS (Mother unaffected)	[37]
	p.Phe139fs (predicted) (12)	c.1007+1G>A	Het		ATD			LKS + MR	[30]
		c.1007+1G>A	Het	Inherited				ABPE + Delayed cognition	[30]
		c.1007+1G>A	Het	Inherited				BECTS + Delayed cognition	[30]
		c.1007+1G>A	Het	Inherited				CSWSS (proband + father)	[38]
		c.1007+1G>A		Inherited				RE (proband + six relatives)	[38]
		c.1007+1G>T						ABPE/CSWSS	[30]
	p.Val375fs (7)	c.1123–2A>G	Het	Inherited	ATD			CSWSS (proband + brother); CSWSS + dysphasia (aunt); LKS (uncle); RE + dysphasia (grandmother); RE (mother, cousin)	[33]
	Not known (3)	c.2007+1G>A	Het	Inherited				CSWSS (proband); Uncharacterized epilepsy (father)	[30]
		c.2007+1G>A		Inherited				CSWS + Moderate LD (Father unaffected)	[36]

Table 2 continued

GRIN2A	Protein (cases)	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
Nonsense (8)	p.Trp198*	c.594G>A	Het		ATD			ABPE + Delayed cognition	[30]
	p.Gln218* (3)	c.652C>T	Het	Inherited	ATD			CTS + moderate ID + MR (proband); FS + focal seizures + learning difficulties (mother); IS + learning difficulties (grandmother)	[29]
	p.Leu334* (3)	c.1001T>A	Het	Inherited	ATD			CSWSS (proband), Panayiotopoulos syndrome (brother), partial epilepsy (father)	[30]
	p.Trp606*	c.1818G>A	Het		M2			ABPE + Mild LD	[36]
	p.Arg681*	c.2041C>T	Het	Inherited	LBD (S2)			LKS + learning disability (proband); learning disability (2)	[30]
	p.Glu803*	c.2407G>T	Het	*De novo*	S2-M4 linker			LKS + Moderate LD	[36]
	p.Tyr943* (3)	c.2829C>G	Het	Inherited	CTD			CSWSS (proband) + MR; FS + CTS (sister); RE (father)	[30]
	p.Tyr1387* (3)	c.4161C>A		Inherited	CTD			CSWSS + Autistic features (proband); BCE (mother, uncle)	[33]
Missense (50)	p.Met1Thr (3)	c.2T>C		Inherited	ATD			LKS (proband, sister); Seizures + speech/language impairment (father)	[38]
	p.Pro31Thr	c.91C>A			ATD			BECTS	[30]
	p.Pro79Arg (4)	c.236C>G	Het	Inherited	ATD	Glu↓, Gly↓, Total protein↓, Surface expression↓	LOF	CSWSS (proband) + Severe attention deficits; BECTS (mother, uncle, grandmother)	[30, 39]
	p.Thr141Met	c.422C>T	Het		ATD			TLE	[40]
	p.Phe183Ile	c.547T>A	Het	Inherited	ATD			BECTS + Delayed cognition	[30]
	p.Ile184Ser	c.551T>G		Inherited	ATD	Current ↓, Activation time↑, Deactivation time↑, Surface expression↓	LOF	CSWSS	[33, 41]
	p.Cys231Tyr (3)	c.692G>A	Het	Inherited	ATD	Glu↓, Gly↓, Total protein→, Surface expression↓	LOF	LKS + ID (proband); CTS (brother, sister)	[30, 39]
	p.Ala243Val	c.728C>T	Het		ATD	Glu→, Zn^{2+}↓	GOF	BECTS + Learning problems	[30]
	p.Ala290Val	c.869C>T	Het		ATD			BECTS	[30]
	p.Gly295Ser	c.883G>Ac			ATD			RE	[33]
	p.Arg370Trp	c.1108C>T	Het		ATD			BECTS	[30]

Table 2 continued

GRIN2A	Protein (cases)	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
	p.Cys436Arg	c.1306T>C	Het	*De novo*	LBD (S1)	Current↓, Glu↑, Gly↓, Total protein↓, Surface expression↓	LOF	ABPE	[30, 39, 42]
	p. Val452Met	c.1354G>A			LBD (S1)	Current→, Glu↑, Gly→, τ_w→, Total protein→, Surface expression→	GOF	Schizophrenia (2)	[42, 43]
		c.1354G>A	Het	*De novo*	LBD (S1)			Intractable seizures	[44]
	p.Gly483Arg (2)	c.1447G>A		Inherited	LBD (S1)	Current→, Glu↓, Gly↓, τ_w ↓, Total protein↓, Surface expression↓	LOF	CSWSS + dysphasia (proband); RE + dysphasia (sister)	[33, 39, 42]
	p.Arg504Trp (3)	c.1510C>T		Inherited	LBD (S1)	Current→, Glu→, Gly→, τ_w↑, Total protein↓, Surface expression↓	LOF	CSWSS (proband, father); FS	[33, 42]
	p.Val506Ala			Inherited	LBD (S1)	Current→, Glu↑, Gly→, τ_w→, Total protein→, Surface expression↑	LOF	Focal seizures	[34, 42]
	p.Arg518His (4)	c.1553G>A		Inherited	LBD (S1)	Current↓, activation time↑, deactivation time↑, Surface expression↓, Single-channel open time↑, close time ↓	LOF	CSWSS (proband); RE (brother); VD (father); CTS	[33, 41, 42]
		c.1553G>A						LKS	[45]
	p.Thr531Met (4)	c.1592C>T		Inherited	LBD (S1)	Current↓, Single-channel open time↑	LOF	CSWSS (proband); Epilepsy-aphasia (two brothers); Learning difficulties + speech/language impairment (mother)	[42, 46]
	p.Ala548Thr	c.1642G>A		*De novo*	S1-M1 linker	Current↓, Glu ↓, Gly ↓, Total protein→, Surface expression→	LOF	LKS	[26, 33]
	p.Pro552Arg	c.1655C>G			S1-M1 linker	Current→, Glu↑, Gly↑, τ_w↑, Total protein→, Surface expression→	GOF	Early-onset seizures + Severe ID + No speech	[26, 47]
	p.Arg586Lys (2)	c.1757G>A	Het	Inherited	M1-M2 linker		No change	Severe EE (parents unaffected)	[29]
		c.1757G>A	Het	Inherited				IS (father unaffected)	[37]

Table 2 continued

GRIN2A	Protein (cases)	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
		c.1757G>A				Current→, Mg^{2+}→, Ifenprodil→, Single-channel conductance→, open time→	ID		[48]
	p.Asn614Ser	c.1841A>G	Het	*De novo*	M2			Focal epilepsy + Severe LD	[36]
	p.Asn615Lys	c.1845C>A	Het	*De novo*	M2	Mg^{2+}↓	GOF	EOEE + Severe MR	[29, 49]
	p.Thr646Ala	c.1936A>G	Het	*De novo*	M3			EE	[36]
	p.Leu649Val	c.1945C>G			M3			Epileptic seizures + Severe ID	[47]
	p.Phe652Val	c.1954T>G		*De novo*	M3	Single-channel open time↑, close time↓	GOF	CSWSS + Autistic features	[33]
Missense (50)	p.Lys669Asn	c.2007G>T		*De novo*	LBD (S2)	Current→, Glu↑, Gly↑, τ_w↑, Total protein→, Surface expression→	GOF	CSWSS	[33, 42]
	p.Val685Gly	c.2054T>G			LBD (S2)	Current↓, Glu↓, Gly→, τ_w↓, Total protein↓, Surface expression↓	LOF	Severe intractable epilepsy+ DD	[42]
	p.Ile694Thr	c.2081T>C		*De novo*	LBD (S2)	Current↓, Glu↓, Gly→, τ_w→, Total protein↓, Surface expression↓	LOF	LKS	[33, 42]
	p.Pro699Ser	c.2095C>T	Het	*De novo*	LBD (S2)	Current→, Glu↑, Gly→, τ_w→, Total protein→, Surface expression↓	GOF	BECTS	[30, 42]
	p.Met705Val	c.2113A>G	Het	Inherited	LBD (S2)	Current→, Glu↓, Gly→, τ_w→, Total protein→, Surface expression↓	LOF	BECTS	[30, 39, 42]
	p.Glu714Lys	c.2140G>A	Het		LBD (S2)	Current→, Glu→, Gly→, τ_w→, Total protein↓, Surface expression↓	LOF	CSWSS	[30, 39, 42]
	p.Ala716Thr (8)	c.2146G>A		Inherited	LBD (S2)	Current→, Glu↓, Gly→, τ_w ↓, Total protein↓, Surface expression↓	LOF	RE + VD (proband + six relatives); VD (cousin)	[33, 41, 42]
		c.2146G>A	Het					LKS +LD + Diffuse hypotonia + Lack of motor coordination.	[50]
	p.Ala727Thr	c.2179G>A	Het		LBD (S2)	Current→, Glu↓, Gly→, τ_w→, Total protein↓, Surface expression↓	LOF	BECTS	[30, 42]
	p.Asp731Asn (3)	c.2191G>A	Het	*De novo*	LBD (S2)	Glu↓, H^+↑,Zn^{2+}↑,τ_w↓ , P0↓	LOF	Unexplained epilepsy + DD	[42, 51]
		c.2191G>A		*De novo*				LKS	[52]
		c.2191G>A		Inherited		Current↓, Glu↓, Gly↓, Total protein↓, Surface expression↓		RE + VD (proband); VD (mother)	[33, 39, 42]
	p.Val734Leu (2)	c.2200G>C	Het	Inherited	LBD (S2)	Current→, Glu↓, Gly→, τ_w↓, Total protein→, Surface expression→	LOF	BECTS (proband, brother)	[30, 42]
	p.Lys772Glu	c.2314A>G	Het		LBD (S2)	Current→, Glu↓, Gly→, τ_w→, Total protein↓, Surface expression↓	LOF	ABPE + Learning and reading problems	[30, 42]
	p.Leu812Met	c.2434C>A	Het	*De novo*	S2-M4 linker	Current→, Glu↑, Gly↑, Mg^{2+}↓, H^+↓,Zn^{2+}↓, τ_w↓, P_0↑	GOF	EOEE + DD	[72]

Table 2 continued

GRIN2A	Protein (cases)	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
	p.Ile814Thr	c.2441T>C	Het	Inherited	S2-M4 linker	Glu→, Gly→, Total protein→, Surface expression→	No change	BECTS	[30, 39]
	p.Met817Val (2)	c.2449A>G		*De novo*	M4	Current→, Glu↑, Gly↑, Mg^{2+}↓, H^{+}↓, Zn^{2+}↓, τ_w↓, P0↑	GOF	Refractory epilepsy + DD	[53, 54]
		c.2448C>T		*De novo*	M4			Unexplained epilepsy + ID	[52]
	p.Ile876Thr	c.2627T>C	Het		CTD			TLE	[40]
	p.Ile904Phe (3)	c.2710A>T	Het	Inherited	CTD			BECTS + Delayed cognition (proband, father); FS + CTS (brother)	[30]
	p.Asp933Asn	c.2797G>A		Inherited	CTD	Glu→, Gly→, Total protein→, Surface expression→	No change	LKS	[33, 39]
	p.Val967Leu (12)	c.2899G>C	Het		CTD			TLE	[40]
		c.2899G>C			CTD			ABPE (2); ABPE/unclassified epilepsy; BECTS (8)	[30]
	p.Asn976Ser	c.2927A>G	Het		CTD	Glu→, Gly→, Total protein→, Surface expression→	No change	ABPE/CSWS	[30, 39]
Missense (50)	p.Thr1064Ala (2)	c.3190A>G			CTD			Schizophrenia	[43]
		c.3190A>G	Het	Inherited				Epilepsy	[29]
		c.3190A>G	Het					BECTS	[30]
	p.Asn1076Lys (3)	c.3228C>G	Het		CTD			BECTS	[29, 30]
		c.3228C>G		Inherited				BECTS	[29, 30]
		c.3228C>G		Inherited				LKS	[29, 30]
	p.Asp1251Asn (2)	c.3751G>A		Inherited	CTD			RE (proband); Absence epilepsy (father)	[33]
	p.Ala1276Gly (3)	c.3827C>G			CTD			CSWSS (2); BECTS	[30, 33]
	p.Ile1379Val	c.4135A>G		*De novo*	CTD			Juvenile Absence Epilepsy	[40]

ATD amino-terminal domain, *ABPE* atypical benign partial epilepsy, *BCE* benign childhood epilepsy, *BECTS* benign epilepsy with centro-temporal spikes, *CSWS* continuous spike and slow-wave during sleep, *CTS* centro-temporalspikes, *DD* developmental delay, *FS* febrile seizures, *GOF* gain-of-function, *GTCS* generalized tonic-clonic seizures, *IS* infantile spasms, *LD* language delay, *LKS* Landau-Kleffner syndrome, *MR* mental retardation, P_0 single-channel open probability, *RE* Rolandic Epilepsy, *TLE* temporal lobe epilepsy, *VD* verbal dyspraxia, τ_w deactivation time course

↑ increase

mutations, the phenotypes of patient relatives in the same family are sometimes different [29, 30, 33] and even one or more members in the family can be unaffected by epilepsy [36, 37]. This is perhaps due to incomplete penetrance. This evidence suggests that some mutations just act as a genetic risk factor conferring susceptibility to epilepsy rather than having a direct pathogenic role.

Interestingly, there are epilepsy-associated homologous mutations in NMDAR subunits. C436R is a mutation existing in both GluN2A and GluN2B at an homologous site. GluN2A and GluN2B C436R both dramatically decrease the peak amplitude of NMDAR currents. Nevertheless, the phenotype of the GluN2B C436R mutation is much more serious than that of the GluN2A C436R mutation [30, 39, 42]. A little different from this situation, GluN2A N615K and GluN2B N615I are both located in the pore region of the NMDAR channel. In N615K, the first asparagine (N) of the NNSVPV sequence is mutated into lysine (K), while in N615I, the second asparagine (N) of the NNSVPV sequence is mutated into isoleucine (I). These two mutations give rise to similar functional changes and have the clinical phenotypes EOEE and West syndrome, respectively [29, 56].

It is unexpected that some mutations are associated with epilepsy as well as other neurological diseases. Some of the epilepsy-associated *GRIN1* mutations also display autism-spectrum disorder (ASD) or ASD-like features [22]. GluN2A V452M is associated with schizophrenia [43] and intractable seizures [44]. Y1387* and F652V mutations have both been identified in patients with CSWS as well as autistic features [33]. Similarly, the missense mutation C461F [42] and the 12p13.1q21.31 inversion mutation [57] in *GRIN2B* are both associated with epilepsy and autism. These lines of evidence remind us that one mutation may have the potential to cause more than one kind of neurological disease or that some neurological diseases have internal links.

Mutations located at the GluN2A LBD have variable phenotypes ranging from mild BECTS to severe LKS and mutations located at different domains of the NMDAR subunits can display the same or similar phenotype, suggesting that location has nothing to do with the phenotype (Table 2). However, the c.2449A>G, p.M817V mutation is refractory epilepsy with developmental delay, while the c.2448C>T, p.M817V mutation is unexplained epilepsy with ID [52, 53], suggesting that the property of the nucleotide at the mutation site might influence the clinical phenotype.

Diversity of Functional Consequences

Epilepsy-associated NMDAR subunit mutations bring about multiple functional consequences. Among all the mutations that have been functionally evaluated, only six out of forty-six were found to have no effect on the function of NMDARs, the remaining forty mutations all having functional changes, supporting the pathogenicity of NMDAR mutations in epilepsy (Tables 1–4). Currently, most of the mutations that have been functionally investigated are missense mutations located in the extracellular domain and the TMD, and only three mutations located in the C-terminal have been tested (GluN1 R844C, and GluN2A D933N and N976S), but no change was found (Table 5). The missense mutations located in the LBD have all been functionally evaluated except for GluN1 S688Y and GluN2B Q662P.

It is worth mentioning that six *GRIN1* mutations (S560dup, Q556*, D552E, Y647S, G815R, and G827R), six *GRIN2A* mutations (I184T, C436R, R518H, T531M, V685G, and D731N), and two *GRIN2B* mutations (C436R and C461F) identified in epilepsy lead to almost complete loss of the NMDAR current [22, 41, 42], thus pointing out the importance of these amino-acids at corresponding sites in NMDAR function.

In general, based on the functional consequences, mutations can be classified into three types: gain-of-function, loss-of-function, and no change. Mutations with loss-of-function effects are the most common form (Table 4). These gain-of-function and loss-of-function mutations can change NMDAR properties in different aspects, including current density, glutamate and glycine potency, sensitivity of the magnesium, zinc, proton, and synaptic-like response time course (τ_w), single-channel opening time and open probability, and total and surface receptor expression levels. Different mutations may cause different or similar changes. Some mutations alter one or a few properties of NMDARs [29, 30], and some cause comprehensive changes [54, 72]. Not all the properties are changed consistently. For example, changes in NMDAR current density and protein expression level are sometimes contradictory [42, 56]. Such inconsistency even exists in agonist sensitivity. For instance, GluN2B C461F causes reduced glutamate potency as well as enhanced glycine potency [42]. To evaluate the overall functional consequences of a mutation, the first step is to examine the current density. If the current density of a mutant is the same as the wild-type NMDARs, then it is necessary to test properties such as expression level and sensitivity to magnesium, zinc, calcium, and protons.

The functional consequences of *GRIN2A* gene mutations seem to be more diverse than other NMDAR subunit mutations (Tables 1–3). The large number and the many modulatory sites may contribute to this diversity. The properties of amino-acid residues at the same site may be one of the factors that affect NMDAR function [26, 72]. GluN2A L812M is a good example. Leucine located at 812

Table 3 Summary of *GRIN2B* mutations identified in epilepsy

GRIN2B	Protein	Gene	Zygosity	Origin	Location	Functional validation	Consequences	Phenotype	References
Deletion (2)	Not known	12p13.1 microdeletion		*De novo*				Epilepsy + ID + DD	[56]
	p.Phe671_Gln672del	c.2011-5 2011-4delTC		Inherited	LBD (S2)			West syndrome	[56]
Inversion (1)	Not known	inversion (12) (p13.1q21.31)		*De novo*	-			Epilepsy + ID + ASD	[57]
Splice-site (1)	Not known	NM_000834.3:c.2011-1G>A	Het	*De novo*				EOEE	[58]
Missense (9)	p.Glu47Gly	c.140A>G		Inherited	ATD			TLE	[59]
	P.Glu370Lys	c.1108G>A			ATD			TLE	[59]
	p.Cys436Arg	c.1306T>C		*De novo*	LBD (S1)	Current↓, Total protein↓, Surface expression↓	LOF	Epilepsy + ID	[42]
	p.Cys461Phe	c.1382G>T		*De novo*	LBD (S1)	Current↓, Glu↓, Gly↑, τ_w↓, Total protein↓, Surface expression↓	LOF	LGS + ID + ASD	[27, 42]
	p.Arg540His	c.1619G>A	Het	*De novo*	LBD (S1)	Current→, Glu↑, Gly↑, τ_w↑, Mg^{2+}↓, Total protein↓, Surface expression↓,	GOF	Focal epilepsy + ID	[42, 56, 60]
	p.Asn615Ile	c.1844A>T	Het	*De novo*	M2	Glu→, Mg^{2+}↓, Ca^{2+}↑	GOF	West Syndrome + severe DD	[56, 60]
	p.Val618Gly	c.1853T>G	Het	*De novo*	M2	Glu→, Mg^{2+}↓, Ca^{2+}↑	GOF	West Syndrome + severe DD	[56, 60]
	p.Gln662Pro	c.1985A>C		*De novo*	LBD (S2)			IS + Myoclonus + severe ID	[61]
	p.Met824Arg	c.2471T>G		*De novo*	M4			Epilepsy + ID + DD	[62]

ASD autism spectrum disorder

Table 4 Summary of NMDAR subunit mutations in epilepsy

Gene	Number	Functional validation			
		Gain-of-function	Loss-of-function	No change	Sum
GRIN1	12	0	6	2	8
GRIN2A	82	9	19	4	32
GRIN2B	13	3	2	0	5
GRIN2D	1	1	0	0	1
Sum	108	13	27	6	46

is critical for NMDAR function and any substitution leads to over-activation of NMDARs [72].

Gain-of-function mutations of NMDARs may cause over-excitation of the brain, which could potentially give rise to epilepsy. For the loss-of-function mutations identified in the *GRIN2A* and *GRIN2B* genes, disruption of the inhibitory network and neural development might account for their pathogenicity in epilepsy [73]. Furthermore, *GRIN2A* loss-of-function mutations might activate compensatory mechanisms that induce more GluN2B-containing NMDAR expression, which would lead to a longer opening time of NMDARs and thus result in temporarily hyperactive NMDARs and excitotoxicity [74].

The pathogenicity of those mutations that do not change NMDAR function in epilepsy is unknown. It is likely that they do not play a causative role, however, we also cannot exclude the possibility of pathogenicity, as these mutations might affect undetected downstream signaling, or disturb the inhibitory network, which might lead to epilepsy as well.

Of course, not all mutations identified in epilepsy are pathogenic. Some mutations might just increase the susceptibility to epilepsy as they also occur in healthy controls. Environmental factors, genetic background, and other elements can also contribute to epilepsy. Furthermore, as clinicians usually focus on the disease in which they are interested, other concurrent diseases in patients with epilepsy may be ignored in screening. Hence, some mutations may cause diseases other than epilepsy.

Relationship Between Functional Alteration and Phenotype

There are a large number of epilepsy-associated NMDAR subunit mutations and they are distributed in all major domains of the subunit, resulting in NMDAR mutants with diverse functional changes and thus lead to extremely complex clinical manifestations. However, in general, the severity of the functional consequences appears to have no direct relationship with the severity of the clinical phenotypes (Tables 1–3).

The functional consequences of *GRIN1* mutations are very different. Six mutations (D552E, S560dup, Y647S, G815R, Q556*, and G827R) dramatically reduced the function of NMDARs, but the other two mutations, A645S and R844C, appear not to impact receptor channel function (Tables 1, 4). However, the severity of the phenotypes is similar (Table 1). Other undetected secondary physiological changes caused by these mutations rather than direct functional changes of the NMDAR channels might contribute to this inconsistency [22].

Such inconsistency also exists in epilepsy-associated *GRIN2A* mutations. According to functional studies, BECTS and CSWS are both mainly associated with loss-of-function effects; however, there are also two gain-of-function mutations and one mutation with no functional change. Although no gain-of-function mutation has been discovered in LKS, the loss-of-function effects can be moderate or serious, and one mutation with no change has also been reported in LKS (Table 2).

As few *GRIN2B* mutations have been functionally evaluated, it is difficult to speculate on the relationship between a functional change of the mutant receptor and the clinical phenotype. From the current data, most of the *GRIN2B* mutations result in large functional changes and the epileptic phenotypes are mostly severe. However,

Table 5 Localization of functionally validated NMDAR subunit mutations in epilepsy

	GRIN1	*GRIN2A*	*GRIN2B*	*GRIN2D*	Sum
ATD	0	4	0	0	4
LBD-S1	0	7	3	0	10
S1-M1 linker	3	3	0	0	6
TM1	0	0	0	0	0
TM2	0	1	2	0	3
TM3	2	1	0	1	4
M3-S2 linker	0	0	0	0	0
LBD-S2	0	11	0	0	11
S2-M4 linker	0	2	0	0	2
TM4	2	1	0	0	3
CTD	1	2	0	0	3
Sum	8	32	5	1	46

R540H, which only leads to mild functional changes and mild phenotypes, is an exception (Table 3).

Of course, similar functional changes can lead to similar phenotypes. The GluN2A mutations R518H and T531M were separately identified in two sporadic CSWS cases. These mutations both decrease the current amplitude and increase the single-channel opening time of the NMDAR [33, 46]. The same phenomenon is also found in the GluN2B subunit. The N615I and V618G mutations were separately identified in two unrelated patients with infantile spasm. These mutations both significantly reduce the inhibitory effect of magnesium on NMDARs and increase the permeability of calcium through the receptor [56].

Treatment

The dramatically increasing number of known NMDAR subunit mutations in epilepsy suggests that NMDARs can serve as an important molecular target for epilepsy therapy [3, 5, 75]. Anti-epileptic drugs usually control the seizures easily in mild epileptic types, such as BECTS and focal epilepsy of childhood. However, it is often difficult to cure more severe seizure types like epileptic encephalopathy with continuous spike-wave during sleep. Intriguingly, a patient with EOEE carrying the GluN2A L812M mutation exhibited resistance to various anti-epileptic drugs such as lacosamide, rufinamide, and valproic acid, whereas memantine, a non-competitive antagonist of the NMDAR, improved his condition [38, 72]. Memantine is an FDA-approved drug used clinically for the treatment of Alzheimer's disease. This case indicates that it is promising to screen drugs among FDA-approved NMDAR antagonists for personalized epilepsy therapeutics. Hence, ketamine, magnesium, dextromethorphan, dextrorphan, amantadine, tomoxetine, and TCN-201 have been tested by electrophysiological experiments *in vitro*. These drugs effectively inhibit the current of NMDARs containing the GluN2A-L812M, GluN2A-M817V, and GluN2D-V667I mutations in a dose-dependent manner, and are thought to be promising personalized drugs for epilepsy [54, 63, 72]. However, in some intractable cases, treatment with one drug alone is not enough. GluN2D V667I was identified in two unrelated children with epileptic encephalopathy. One of them was refractory to memantine, midazolam, pentobarbital, ketamine or magnesium alone. However, ketamine in combination with the magnesium infusion dramatically improved this patient's condition. The other was refractory to memantine alone and finally was controlled by a combination of memantine, sulthiame, and lamotrigine [63]. Therefore, for intractable epilepsy such as epileptic encephalopathy, the combined use of anti-epileptic drugs and/or FDA-approved NMDAR antagonists may be a good choice. Recently, radiprodil, a negative allosteric modulator of GluN2B-containing NMDARs, was demonstrated to be as effective on GluN2B R540H, N615I, and V618G mutants as on wild-type NMDARs by *in vitro* experiments [60]. Therefore, radiprodil may be a valuable therapeutic option for treatment of pediatric epileptic encephalopathies associated with *GRIN2B* mutations.

Epilepsy-associated NMDAR subunit mutations produce a variety of functionally altered receptor mutants and diverse epileptic clinical phenotypes, while different patients respond differently to anti-epileptic drugs. Hence, personalized treatment for patients with epilepsy carrying NMDAR mutations requires further work.

Conclusions and Perspectives

In this review, we summarize the NMDAR subunit mutations associated with epilepsy, including their genotypes, properties of functional alterations of the mutant receptor channels, and clinical phenotypes. We also tried to analyze the possible relevance of genotypes with functional changes and clinical manifestations. The information is expected to provide clues in assessing the pathogenicity of these *GRIN* mutations, and could lead to mechanistic insights into the roles of NMDARs in epilepsy and precision treatments in the future.

So far, most of the epilepsy-associated NMDAR mutations that have been functionally investigated are located in the extracellular domain and TMD, and are limited to missense mutations (Tables 1, 2, 3, 5). This lack of functional analysis of mutations located in the C-terminal could be due to difficulty and limitations in detecting direct changes in NMDARs using current approaches. In order to better understand the subtle and complicated actions of NMDARs in human epilepsy and to develop precision therapeutics, more functional studies of epilepsy-related NMDAR mutations are needed. However, since *in vitro* experiments are not suitable for detecting the exact physiological changes, the field is limited to analyzing the correlation of phenotypes and genotypes. Further studies using knock-in mice and *in vivo* experiments are needed to confirm the pathogenicity of the mutations identified in epilepsy.

As some *GRIN* mutations have been repeatedly identified in epilepsy and some result in similar functional changes and the same clinical phenotypes, the establishment of a database composed of information on "epilepsy-related NMDAR mutation—functional alteration—phenotype—drug treatment" would provide better guidance and offer greater convenience in genetic diagnosis and precision therapeutics for epilepsy. However, the number of known NMDAR mutations in epilepsy is increasing continuously, and they lead to multiple functional

consequences, diverse epileptic phenotypes, and different drug tolerances. Therefore, precision treatment for epilepsy with causative mutations of NMDARs is full of challenges and needs long-term unrelenting effort.

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