Clinical Implications of Basic Neuroscience Research

Molecular Pathogenic Basis for *GABRG2* Mutations Associated With a Spectrum of Epilepsy Syndromes, From Generalized Absence Epilepsy to Dravet Syndrome

Jing-Qiong Kang, MD, PhD; Robert L. Macdonald, MD, PhD

OBJECTIVE In this review article, we focus on the molecular pathogenic basis for genetic generalized epilepsies associated with mutations in the inhibitory γ-aminobutyric acid ($GABA_A$) receptor γ2 subunit gene, *GABRG2* (OMIM 137164), an established epilepsy gene.

OBSERVATIONS The γ-aminobutyric acid ($GABA_A$) receptor γ2 subunit gene, *GABRG2*, is abundantly expressed in the mammalian brain, and its encoded γ2 subunit is assembled into αβγ2 receptors, which are the major $GABA_A$ receptor isoforms in the brain. The γ2 subunits have a critical role in $GABA_A$ receptor trafficking and clustering at synapses. They reside inside the endoplasmic reticulum after synthesis, where they oligomerize with other binding partners, such as α and β subunits, and further assemble into pentameric receptors. Only correctly assembled receptors can traffic beyond the endoplasmic reticulum and reach the cell surface and synapses, where they conduct chloride ion current when activated by GABA. Mutations in *GABRG2* have been associated with simple febrile seizures and with genetic epilepsy syndromes, including childhood absence epilepsy, generalized epilepsy with febrile seizures plus, and Dravet syndrome or severe myoclonic epilepsy in infancy. The mutations include missense, nonsense, and frameshift mutations, as well as splice-site and deletion mutations. The mutations have been identified in both coding and noncoding sequences like splice sites. In the coding sequence, these mutations are found in multiple locations, including the extracellular N-terminus, transmembrane domains, and transmembrane 3–transmembrane 4 intracellular loop. All of these mutations reduced channel function but to different extents and by diverse mechanisms, including nonsense-mediated messenger RNA decay, endoplasmic reticulum–associated protein degradation, dominant negative suppression of partnering subunits, mutant subunit aggregation causing cell stress and cell death, and gating defects.

CONCLUSIONS AND RELEVANCE We conclude that the epilepsy phenotypic heterogeneity associated with *GABRG2* mutations may be related to the extent of the reduction of $GABA_A$ receptor channel function and the differential dominant negative suppression, as well to toxicity related to the metabolism of mutant subunit proteins resulting from each mutant γ2 subunit, in addition to different genetic backgrounds.

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Genetic epilepsies refer to epilepsy syndromes previously classified as idiopathic generalized epilepsies and have been associated with gene mutations using current advances in genetics and sequencing technology.[1] There are many ion channel and non–ion channel gene mutations that have been identified in epilepsy.[2] These ion channel genes include those encoding proteins that form channels conducting sodium, potassium, calcium, and chloride ions. Those non–ion channel genes include an overwhelmingly comprehensive and still growing list of genes, with their function involved in protein endocytosis, synaptic development, and transcription, as well as many unknown mechanisms. To date, most functional studies of epilepsy genetic mutations have been focused on ion channel genes. Mutation of ion channels that cause either a "gain of function" in excitatory neurotransmission or a "loss of function" in inhibitory neurotransmission could impair the balance of excitation and inhibition, leading to disinhibition and hyperexcitability in the brain. In this review article, we focus on the molecular pathogenic basis for genetic generalized epilepsies associated with mutations in the inhibitory γ-aminobutyric acid ($GABA_A$) receptor γ2 subunit gene, *GABRG2* (OMIM 137164), an established epilepsy gene that we have extensively characterized.

Generalized epilepsies (GEs) are the most common neurological disorders in the pediatric population and one of the most common neurological disorders in adults.[3] They include several different epilepsy syndromes that vary in clinical severity from benign childhood absence epilepsy (CAE), which may remit with age, to more severe juvenile myoclonic epilepsy and generalized epilepsy with febrile seizures plus (GEFS+). A subpopulation of GEs are associated with severe recurrent seizures and cognitive decline that have been referred to as epileptic encephalopathies, which include severe myoclonic epilepsy in infancy or Dravet syndrome, West syndrome or infantile spasms, Ohtohara syndrome, and Lennox-Gastaut syndrome.

GEs and $GABA_A$ Receptors

The $GABA_A$ receptors are the primary mediators of fast inhibitory synaptic transmission in the central nervous system and have been repeatedly documented to have a critical role in animal models of seizures.[4-11] These inhibitory receptors are heteropentamers formed by assembly of multiple subunit subtypes (α1-α6, β1-β3, γ1-γ3, δ, ε, π, θ, and ρ1-ρ3). These receptors form chloride ion channels and most commonly contain 2 α subunits, 2 β subunits, and a γ or δ subunit. The $GABA_A$ receptors mediate both phasic, inhibitory synaptic transmission and tonic, perisynaptic inhibition, and several antiepileptic drugs, including benzodiazepines, barbiturates, and neurosteroids, act by enhancing $GABA_A$ receptor channel currents.[12] All $GABA_A$ receptor subunits share a similar protein topology, which contains a large extracellular N-terminus, 4 transmembrane domains, a small extracellular loop between the second and third transmembrane domains, and a larger intracellular loop between the third and fourth transmembrane domains.

It is important to understand the normal trafficking route of $GABA_A$ receptor subunits to understand the molecular defects resulting from the receptor subunit mutations (**Figure 1**). All $GABA_A$ receptor subunits are transported to the endoplasmic reticulum (ER) after synthesis and signal peptide cleavage. Therefore, the mature peptide that will be assembled into pentameric receptors will not contain the signal peptide. In the ER, the subunit first oligomerizes with its binding partners, forming homodimers or heterodimers like α-α dimers or α-β dimers.[13] These dimers further assemble into a pentamer with other subunits. Those unassembled or misfolded subunits, including both wild-type and mutant subunits, are subject to ubiquitin-proteasome system degradation. Only correctly assembled receptors can traffic beyond the ER to the *trans*-Golgi and endosomes to finally reach the cell membrane and synapses. Only those subunits that reach the membrane surface and synapses can mediate inhibition by conducting chloride ions, while those subunits residing in intracellular compartments have no function (Figure 1).

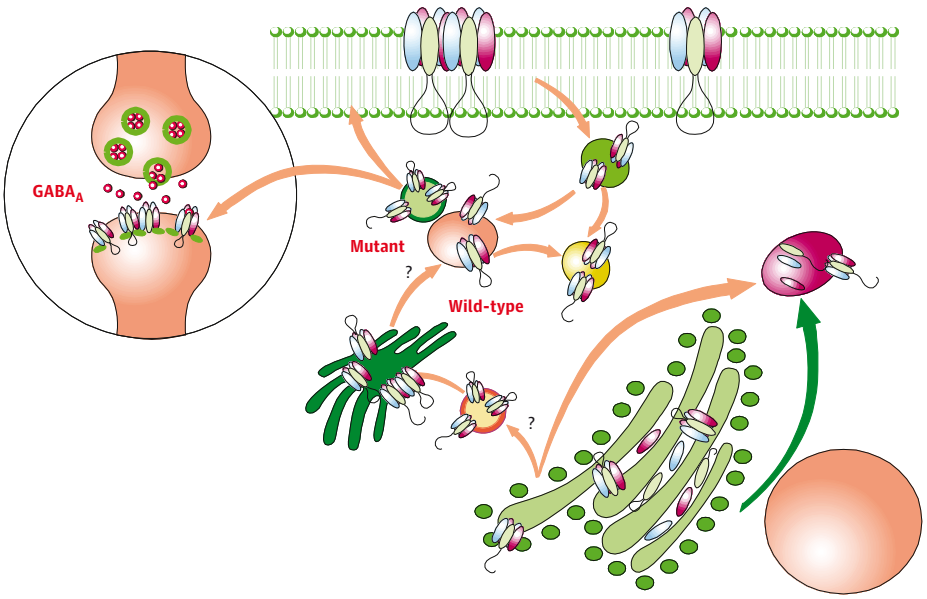
The $GABA_A$ receptor subunits form a superfamily that contains 19 subunits. Mutations or variants in several $GABA_A$ subunits have been associated with epilepsies. These subunit genes include *GABRA1*, *GABRB1*, *GABRB2*, *GABRB3*, *GABRG2*, and *GABRD*.[14,15] Most of these mutations have autosomal dominant inheritance; therefore, the patients are heterozygous for the mutation. The seizures and epilepsy syndromes resulting from mutations in these $GABA_A$ receptor subunit genes include multiple GE syndromes and vary in severity. They include pure febrile seizures[16] and epilepsy syndromes, such as CAE[17], and mixed afebrile seizures and febrile seizures (CAE and febrile seizures and GEFS+, including Dravet syndrome) and afebrile seizures.[18-23] The epilepsy mutations include missense, nonsense, insertion, or deletion mutations resulting in frameshift mutations in coding regions, as well as mutations in noncoding regions.

GABRG2 Subunit and Its Epilepsy Mutations

Among all the *GABR* genes, mutations in *GABRG2* have been most frequently associated with GEs, and 11 epilepsy mutations in *GABRG2* have been identified to date (**Figure 2**). It is not surprising that *GABRG2* is an epilepsy gene given its critical role in $GABA_A$ receptor trafficking and its importance for formation of high-conductance $GABA_A$ receptor channels. The $GABA_A$ receptors mediate most fast inhibitory neurotransmission. The γ2 subunit encoded by *GABRG2* is required for postsynaptic $GABA_A$ receptor clustering.[24] Although the γ2 subunit is not required for pentameric receptor assembly,[25] it has been demonstrated that γ2 subunits are favored over β subunits in receptor assembly and that incorporation of γ2 subunits into receptors significantly increases channel conductance.[26] In addition, γ2 subunit–containing receptor channels adopt unique properties by conferring sensitivity to benzodiazepines and insensitivity to zinc.[27] Homozygous *Gabrg2* knockout mice are not viable, while heterozygous *Gabrg2* knockout mice are viable, display anxiety,[28] and are reported to have absence seizures in some genetic backgrounds.[29] Two *Gabrg2* knockin mice (R82Q[30] and Q390X[31]) displayed behavioral seizures, further validating the critical role of the subunit in epilepsy.

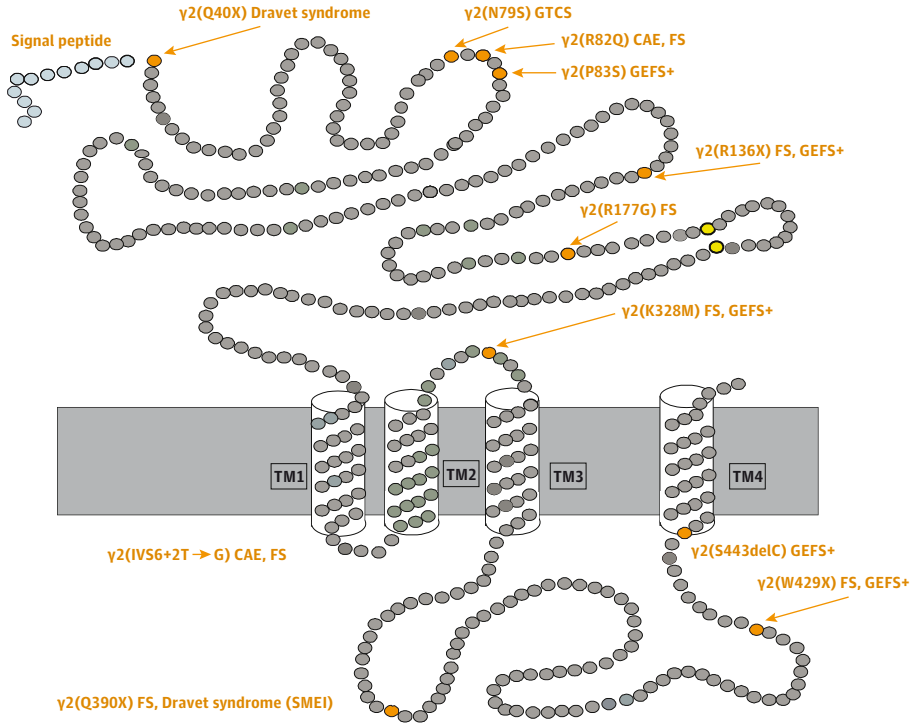
Multiple mutations in *GABRG2* have been associated with epilepsy syndromes with different severities (**Figure 3**). Some mutations in *GABRG2* are associated with simple febrile seizures or CAE, with good outcomes, while others are associated with the more severe GEFS+ phenotype, which continues into adulthood. Still other mutations have even worse phenotypes like Dravet syndrome, with intractable seizures and cognitive decline. These mutations occur in different locations, including the N-terminus, transmembrane domain, intracellular and extracellular loops, and splice donor sites. Mutations in *GABRG2* include missense, nonsense, and deletion or frameshift mutations. Each mutation results in different molecular defects by different molecular mechanisms as detailed below (**Table**).

Figure 1. Schematic Representation Showing the γ-Aminobutyric Acid ($GABA_A$) Receptor Subunit Biogenesis, Assembly, and Trafficking



Shown is the normal trafficking route of $GABA_A$ receptors. Only those receptors that reach the cell surface and synapses can conduct chloride ions and have function, while those subunits residing in intracellular compartments have no function. The mutant subunits (mutant) resulting from missense or nonsense *GABRG2* mutations are subject to nonsense-mediated messenger RNA decay or endoplasmic reticulum–associated degradation. Therefore, the mutant subunits are unlikely to be present on the cell surface and in synapses, as are wild-type receptors. The arrows show the targeted subcellular locations of wild-type or mutant subunits.

Figure 2. Schematic Representation of an γ-Aminobutyric Acid ($GABA_A$) Receptor Subunit Topology Showing the Location of the Epilepsy Mutations in *GABRG2* Identified by Different Groups to Date

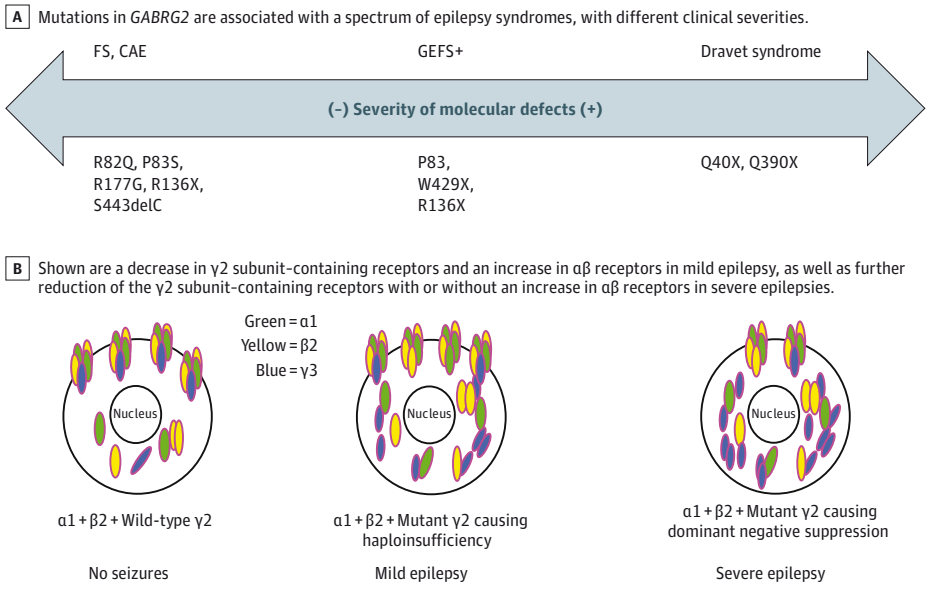


GABRG2 mutations are associated with seizures and epilepsy syndromes. Shown are the γ2 subunit protein and the mutations identified in *GABRG2* to date. CAE indicates childhood absence epilepsy; FS, febrile seizures; GEFS+, generalized epilepsy with febrile seizures plus; GTCS, generalized tonic-clonic seizures; TM, transmembrane; and SMEI, severe myoclonic epilepsy in infancy.

GABRG2 Missense Mutations and Genetic Epilepsy

There are 5 missense mutations in *GABRG2* that have been associated with a genetic epilepsy. There mutations include N79S, R82Q, P83S, R177G, and K328M.[19,20,32,41] Four of the 5 missense mutations (N79S, R82Q, P83S, and R177G) are located in the extracellular N-terminus, and one (K328M) is located in the middle of the short transmembrane 2–transmembrane 3 extracellular linker. The N79S mutation was observed in a single patient with GEFS+. The R82Q and P83S mutations were identified in families with GEFS+. The R177G mutation is associated with CAE, febrile seizures, and GEFS+.

Figure 3. Schematic Representation Showing *GABRG2* Mutations and the Severity of Epilepsy Syndromes



Various clinical phenotypes of seizures and epilepsy syndromes are associated with *GABRG2* mutations. CAE indicates childhood absence epilepsy; FS, febrile seizures; and GEFS+, generalized epilepsy with febrile seizures plus.

Table. *GABRG2* Subunit Mutations and Variants Associated With Genetic Epilepsies and Their Postulated Molecular Defects[a]

GABRG2 Mutation or Variant, Locus 5q34	Postulated Mechanisms	Phenotypes	Source
Missense mutations			
N79S	Impaired oligomerization?	GEFS+	Huang et al,[32] 2014
R82Q	Impaired oligomerization, ER retention	FS, CAE	Wallace et al,[19] 2001
P83S	Impaired oligomerization, ER retention	FS, CAE	Lachance-Touchette et al,[33] 2011
R177G	Impaired oligomerization, ER retention	FS	Todd et al,[34] 2014
K328M	Gating defect	FS, GEFS+	Baulac et al,[20] 2001
Nonsense mutations			
Q40X	NMD, ERAD, ER retention	DS	Huang et al,[35] 2012
R136X	NMD, ERAD	FS, CAE	Johnston et al,[36] 2014
Q390X	ER retention, dominant negative effect	GEFS+, DS	Kang et al,[37] 2009
W429X	ER retention, dominant negative effect?	GEFS+	Kang et al,[38] 2013
Splice-site mutation			
IVS6 + 2T->G	NMD, ERAD	CAE, FS	Tian and Macdonald,[39] 2012
Deletion mutation			
S443delC	ERAD, ER retention?	GEFS+	Tian et al,[40] 2013

Abbreviations: CAE, childhood absence epilepsy; DS, Dravet syndrome; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum–associated degradation; FS, febrile seizures; GEFS+, generalized epilepsy with febrile seizures plus; NMD, nonsense-mediated mRNA decay.

[a] All of these mutations reduced channel function.

All of these missense mutations have been characterized, and the 4 N-terminus mutations all reduced receptor surface expression. The R82Q and P83S mutations resulted in approximately a 60% to 90% reduction of the γ2 subunit on the surface, while the N79S mutation resulted in a small reduction (12%) of the γ2 subunit on the surface.[32] The R177G mutation resulted in approximately a 60% reduction of the surface protein when the mutant subunit was expressed homozygously but approximately a 90% reduction when expressed heterozygously.[34] This finding suggests that the mutant subunit was at a disadvantage for receptor assembly in the presence of the wild-type counterpart. The total protein was reduced with the R82Q and P83S mutations but not with the N79S and R177G mutations. However, the mutant γ2(R177G) subunit had arrested glycosylation and was retained inside the ER, which is not functional. The K328M is the only mutation that altered channel current kinetic properties, by shortening the channel open time, without altering receptor trafficking, thus reducing synaptic inhibition.[42]

GABRG2 Nonsense Mutations and Genetic Epilepsy

There are 4 nonsense mutations in *GABRG2* that have been associated with genetic epilepsy, namely, Q40X, R136X, Q390X, and W429X. The Q40X mutation was observed in twin sisters with Dravet syndrome.[35,43,44] The R136X mutation is associated with GEFS+ and other extended phenotypes like eye myoclonia and autistic features.[36] The Q390X mutation is associated with GEFS+, and the proband was diagnosed as having Dravet syndrome.[18] The

W429X mutation was observed in a Chinese family with febrile seizures and GEFS+.[21] Based on in vitro studies, none of the γ2 subunits with nonsense *GABRG2* mutations have any surface expression.[38] However, each mutation results in different amounts of total γ2 subunit protein levels. Compared with the wild-type γ2 subunit, the mutant γ2(Q40X) subunit protein was undetectable, mutant γ2(R136X) and γ2(W429X) subunit protein was reduced, and γ2(Q390X) subunit protein was increased and formed high-molecular-mass protein complexes. All the truncated γ2 subunit proteins were prone to form high-molecular-mass protein complexes but to different extents.[38]

GABRG2 Splice Donor Site Mutation and Genetic Epilepsy

Only 1 splice donor site mutation in *GABRG2* has been associated with epilepsy to date. The *GABRG2* (IVS6 + 2T→G) splice donor site mutation was observed in a small pedigree with CAE and febrile seizures.[23] This intronic splice donor site mutation has been predicted to cause skipping of exon 6 and creation of an exon 5–exon 7 splice junction and generation of a premature translation termination codon (PTC). Intron splice-site mutation is another kind of PTC-generating mutation, in addition to nonsense and deletion or insertion frameshift mutations. Wild-type and mutant *GABRG2* messenger RNA (mRNA) splicing patterns were determined in both bacterial artificial chromosome–transfected HEK293T cells and transgenic mouse brain, and the mutation in both abolished intron 6 splicing at the donor site, activated a cryptic splice site, generated partial intron 6 retention, and produced a frameshift in exon 7 that created a PTC.[23] The resultant mutant mRNA was either degraded partially by nonsense-mediated mRNA decay or translated to a stable, truncated subunit (the γ2-PTC subunit) containing the first 6 *GABRG2* exons and a novel frameshifted 29 amino acids in the C-terminus tail. The γ2-PTC subunit was homologous to the mollusk acetylcholine-binding protein but was not secreted from cells. It was retained in the ER and not expressed on the surface membrane, but it did oligomerize with α1 and β2 subunits. These results suggested that the *GABRG2* mutation IVS6 + 2T→G decreased surface αβγ2 receptor levels, thus reducing GABAergic inhibition.[39]

GABRG2 Deletion Mutation

There is 1 deletion mutation in *GABRG2* that has been identified in epilepsy. A novel c.1329delC in *GABRG2* was observed in a family with mild generalized epilepsy and febrile seizures.[40] The c.1329delC mutation resulted in a subunit γ2S(S443delC) with a modified and elongated C-terminus that is different from that of the wild-type γ2 subunit. The mutation results in a subunit predicted to lose the last 24 C-terminus amino acids and gain 50 amino acids different from those of the natural variant, with consequent lower hydrophobicity of the C-terminus. This deletion mutation is the first $GABA_A$ receptor epilepsy mutation predicted to abolish the natural stop codon and produce a stop codon in the 3′ untranslated region, thus producing an extended subunit peptide. The subunit mRNA should be stable and should produce γ2 subunits with a disrupted fourth transmembrane domain and an extended C-terminus tail. The mutant γ2 subunit was not present at the cell membrane but was retained inside intracellular compartments. The total mutant γ2 subunit protein was reduced, suggesting that the functional $GABA_A$ receptor on the cell surface and synapses is reduced.[39]

Loss or Reduction of Cell Surface Expression of the Mutant Subunit Protein, a Common Abnormality for *GABRG2* Mutations

We have demonstrated that loss of γ2 subunit protein on the cell surface is a common defect for all the missense, nonsense, and other PTC-generating *GABRG2* mutations. The loss-of-function mutations include all the nonsense, deletion, and splice donor site mutations. The mutations that produce severely impaired subunit surface expression include R82Q, P83S, and R177G, while the N79S mutation has mildly impaired surface expression.[32] The mutant γ2 subunits are retained inside the ER, which is where the immature $GABA_A$ receptor subunit resides once synthesized. With glycosylation studies, we have identified all the mutant subunits that have arrested glycosylation. When coexpressed with the wild-type partnering α1 and β2 subunits, the mutant subunits adopt only ER glycosylation that is the core glycosylation for the immature subunits, while the wild-type γ2 subunits have mature glycosylation, suggesting subunit trafficking beyond the *trans*-Golgi to the cell surface. The γ2 subunits with only core glycosylation are retained in the ER, suggesting that they are nonfunctional.

Altered Channel Kinetics, a Rare Molecular Defect for *GABRG2* Mutations

The only *GABRG2* mutation that results in altered channel kinetics is K328M. The mutation is located in the TM2-TM3 extracellular domain and produces functional consequences that are consistent with decreased neuronal inhibition, including a faster deactivation rate for α1β3γ2L(K328M) $GABA_A$ receptors (which predicts shorter-duration inhibitory postsynaptic currents). Significantly decreased mean open times for α1β3γ2L(K328M) $GABA_A$ receptor single channels were recorded, consistent with the accelerated deactivation, because channels would spend less time in the open state for any given opening before eventually unbinding GABA. Channels that were open at the end of the GABA pulse would close faster than wild-type channels.[42] Accelerated decay of synaptic currents produced by α1β3γ2L(K328M) $GABA_A$ receptors has also been observed in neuronal preparations.[45]

GABRG2 Mutations Causing Simple Loss of *GABRG2* Subunit Function

We have compared the simple loss of one-half of *GABRG2* and the mixed wild-type and mutant condition in HEK293T cells, which mimics the condition occurring with heterozygous patients. We found that the surface γ2 subunit expression level with a one-half gene dose is more than half (65%-70%) of the wild-type γ2 subunit level, with a full gene dose when the γ2 subunits were coexpressed with α and β subunits. Although the total γ2 subunit protein level in the one-half gene dose condition was indeed half of the level of the wild-type full gene dose condition, current amplitude of $α1β2γ2S^{(+/-)}$ receptors is larger than the current amplitude produced by the loss-of-function γ2 subunit mutation containing α1β2γ2S mixed or "heterozygous" receptors.[37] This finding suggests favorable assembly or more efficient trafficking of the γ2 subunit in the one-half gene dose condition than the mixed or heterozygous γ2/γ2(Q390X) condition. This result could be due to the greater availability of the partnering subunits like α and β subunits or the chaperones in the one-half gene dose condition compared with the crowded mixed or heterozygous γ2/γ2(Q390X) condition.[31,37]

There are some *GABRG2* mutations that may result in a simple loss of function or almost a simple loss-of-function condition. For example, we have demonstrated that there was no dominant negative suppression by γ2(R136X) subunits on the wild-type partnering subunits like the α1 subunit.[36] These mutations often result in a mutant subunit protein that is readily degraded without much interference of the biogenesis and function of the remaining wild-type subunits. Alternatively, nonsense or PTC-generating mutations could result in nonsense-mediated mRNA decay that eliminates the mutant allele at the mRNA level if the PTCs occur in an early exon and activate nonsense-mediated mRNA decay. The functional consequence of these mutations would be similar to the *Gabrg2*$^{+/-}$ knockout condition, which may represent a simple haploinsufficiency condition.

GABRG2 Mutations Causing Dominant Negative Suppression of the Remaining $GABA_A$ Receptor Function

Some *GABRG2* mutations generate mutant γ2 subunits that cause dominant negative suppression of the wild-type $GABA_A$ receptors, while other mutations cause only simple haploinsufficiency. Still other *GABRG2* mutations may cause mild dominant negative suppression of the wild-type subunits. Using the nonsense *GABRG2* mutations as an example, despite loss of function for all the truncated subunits, we have demonstrated that R136X has no dominant negative effect on the remaining α1β2 subunits and that Q390X has a strong dominant negative suppression of the wild-type subunits, while W429X has a mild dominant negative effect on the remaining α1β2 subunits.[38] Therefore, the degree of dominant negative suppression of each mutant γ2 subunit varies likely depends on the specific structural disturbance of each specific mutation.

Trafficking Deficient Mutant Proteins, Subject to ER-Associated Degradation But With Different Rates

The differential dominant negative suppression is likely correlated with the degradation rate for each protein, which is likely true for both missense and nonsense *GABRG2* mutations. A detailed comparison of γ2 subunit protein degradation with the radiolabeled pulse-chase assay has been done with *GABRG2* nonsense mutations. For PTC-generating mutations, because nonsense-mediated mRNA decay is rarely complete, the remaining mutant transcripts should be translated and generate mutant protein. Similar to mRNA surveillance, at the protein level trafficking deficient mutant subunits are subject to ER protein quality control, leading to ER retention or ER-associated degradation (ERAD) after translation. Previous studies have demonstrated that truncated mutant proteins translated from mutant mRNAs that escape nonsense-mediated mRNA decay are often trafficking deficient, misfolded, and misrouted and consequently are subject to ERAD.[46] The mechanisms by which ERAD targets misfolded proteins include the ubiquitin-proteasome system[47] and the autophagy or lysosome pathway.[48] However, the degradation rate of each subunit and of each subunit harboring different mutations may be different. The relative stability of each mutant subunit may vary with the stability of different subtypes of subunits and with the nature and location of the mutation. We have compared the protein metabolism of 3 nonsense mutations in the transmembrane 3–transmembrane 4 and C-terminus of the subunit. We have identified that the mutation with the most accumulation had the slowest degradation rate, while the mutation with the least accumulation at the steady-state level had the fastest degradation rate.[38] A mutation that has mild dominant negative suppression on the wild-type subunits had slightly enhanced degradation compared with the wild-type subunits.[38]

GABRG2 Mutations Causing Gain of Cellular Toxicity

The cellular toxicity resulting from ion channel epilepsy gene mutations has never been addressed, to our knowledge. It is likely that most of these ion channel gene mutations would not cause cellular toxicity. The epilepsy syndromes associated with these gene mutations may respond well to the anticonvulsant drugs by modulating neurotransmission. However, patients with mutations like *GABRG2(Q390X)* that result in a mutant protein with slow degradation and protein aggregation would not be easily treated with a pure anticonvulsant drug. The misfolded and aggregated protein would lead to ER stress. It is established that sustained ER stress would cause neuronal death. We have demonstrated that the γ2 subunit protein progressively accumulated inside neurons and formed protein aggregates, which costained with active caspase 3 in older *Gabrg2*$^{+/Q390X}$ mice.[31] Although we could not distinguish the wild-type and the mutant γ2 (Q390X) subunit in the mutant mice at the protein level, it is likely that the accumulation was due to the mutant γ2 subunit because the γ2 subunit protein expression in the neurons was almost identical in the young and older wild-type mice. This findings suggests that the mutant γ2(Q390X) subunit protein could cause neuronal death.

Phenotype Differences Between *Gabrg2*$^{+/Q390X}$ Knockin and *Gabrg2*$^{+/-}$ Knockout Mice

The *Gabrg2*$^{+/-}$ knockout mouse has been available for a long time.[49] Homozygous knockout mice are not viable, suggesting a critical role of *GABRG2* for survival. The heterozygous knockout mice were reported to have increased anxiety[28] and absence seizures in DBA/2J mice that have seizure-prone genetic backgrounds.[29] However, no spontaneous generalized tonic-clonic seizures were reported in the *Gabrg2*$^{+/-}$ knockout mice. In contrast, in the heterozygous *Gabrg2*$^{+/Q390X}$ knockin mice that carry the nonsense mutation *GABRG2(Q390X)*, the heterozygous mice in C57/BL/6J, the most seizure-resistant mouse background, had spontaneous generalized tonic-clonic seizures and increased mortality, likely to be seizure-related sudden unexpected death in epilepsy.[31] This finding indicates that phenotype differences exist between the 2 mouse lines carrying 2 different loss-of-function mutations in *GABRG2*, which is consistent with the observation that $GABA_A$ receptor function is more reduced in the mixed γ2/γ2 (Q390X) condition than in the simple one-half gene dose condition.[37]

Epilepsy Phenotypic Heterogeneity of *GABRG2* Mutations

Mutations in *GABRG2* have been associated with a spectrum of seizures and generalized epilepsy syndromes, with phenotypes that range from simple febrile seizures to childhood absence seizures to GEFS+ or Dravet syndrome. These mutations are identified in rare families and in sporadic cases with de novo mutations. These mutations include missense, nonsense, and other PTC-generating mutations like deletion and splice-site mutations. Phenotypes associated with missense mutations in *GABRG2* are mild and include familial CAE and febrile seizures.[16,19,20] Nonsense mutations in *GABRG2* are associated with more severe phenotypes, and the phenotypes range from GEFS+ to Dravet syndrome.

The basis for the more severe epilepsy phenotypes with nonsense *GABRG2* mutations is likely related to the extent of receptor function reduction and the metabolism of the mutant γ2 subunit protein. Among all the loss-of-function mutations generated by different nonsense *GABRG2* mutations, the *GABRG2(Q390X)* mutation that generates more mutant protein is associated with a more severe epilepsy phenotype. The production of more steady-state mutant γ2(Q390X) subunit protein is due to the slow degradation rate of the mutant protein. The mutant γ2(Q390X) subunit protein is stable, accumulated and aggregated inside cells, and had dominant negative suppression, which would further reduce the function of the remaining $GABA_A$ receptors and thus result in a more severe epilepsy phenotype.

Clinical Implications

The recent findings of *GABRG2* epilepsy mutations provided novel molecular targets for potential new therapeutic strategies for treatment of genetic epilepsies. Conventional antiepileptic drugs work primarily by directly or indirectly regulating neurotransmission. Based on the findings from *GABRG2* mutations, potential therapeutic approaches would include increasing wild-type or mutant $GABA_A$ receptor channel function or decreasing disturbance of the cellular signaling by the presence of the mutant $GABA_A$ receptor subunit protein. The drug to enhance $GABA_A$ receptor function would be effective to compensate the lost or impaired $GABA_A$ channel function. For those *GABRG2* mutations that cause dominant negative suppression and cellular toxicity, removing the nonfunctional mutant protein would increase the remaining wild-type $GABA_A$ receptor function and decrease the nonfunctional mutant protein and cellular toxicity.

Conclusions

In summary, recent studies suggest that the pathogenesis of *GABRG2* mutations is likely to be due to a combination of reduction of channel function and disturbance of cellular homeostasis due to the presence of mutant protein. Therefore, a combined therapeutic strategy to enhance the wild-type $GABA_A$ receptor channel function and eliminate the production of mutant protein using RNA interference targeting of the mutant transcripts or small molecules to promote the disposal of the mutant protein might be a useful approach.

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