Published in final edited form as:
Trends Mol Med. 2009 September ; 15(9): 430–438. doi:10.1016/j.molmed.2009.07.003.

Making Sense of Nonsense $GABA_A$ Receptor Mutations Associated with Genetic Epilepsies

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Abstract

Nonsense mutations that generate premature translation-termination codons (PTCs) are responsible for about one-third of human genetic diseases. PTCs in both voltage- and ligand-gated ion channel genes, including sodium, potassium, nicotinic cholinergic receptor and $GABA_A$ receptor channel genes have been associated with genetic epilepsies, but the epilepsy syndromes they cause are variable. We recently proposed that two well-established molecular pathways, nonsense-mediated decay (NMD) and endoplasmic reticulum (ER) associated degradation (ERAD), determine the effects of PTCs in $GABA_A$ receptor subunit genes associated with genetic epilepsies on the cellular fates of mutant subunit mRNAs and proteins. Activation of these different molecular mechanisms may contribute partially to different clinical phenotypes in patients with $GABA_A$ receptor subunit gene PTCs, and thus we suggest that different approaches for treatment of their genetic epilepsies may be required.

Keywords

$GABA_A$ receptor subunits; Nonsense; Premature translation-termination codons (PTCs); Mutation; Epilepsy

Introduction

Genetic epilepsies refer to epilepsy syndromes previously classified as idiopathic generalized epilepsies (IGEs) (Box 1). IGEs affect about 1% of the population worldwide and are one of the most common neurological disorders (1). IGEs include several different epilepsy syndromes that vary in clinical severity from relatively benign febrile seizures (FS) and childhood absence epilepsy (CAE) to more severe juvenile myoclonic epilepsy (JME) and generalized epilepsy with febrile seizures plus (GEFS+). A subpopulation of GEFS+ patients have severe recurrent seizures with cognitive decline that has been referred to as severe myoclonic epilepsy in infancy (SMEI) or Dravet syndrome. The majority of IGEs likely have a genetic component (2,3), and it is becoming increasingly clear that mutations of transmembrane ion channels, including both voltage-gated and ligand-gated ion channels, are the underlying cause of many forms of human epilepsy including those found in large pedigrees and in sporadic cases with *de novo* mutations (Box 1) (3-5). Mutation of ion

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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 we will focus on the molecular pathophysiological basis for IGEs associated with inhibitory $GABA_A$ receptor channel subunit gene nonsense mutations that generate premature translation-termination codons (PTCs).

Genetic epilepsies and $GABA_A$ receptors

$GABA_A$ receptors are the primary mediators of fast inhibitory synaptic transmission in the central nervous system and have been repeatedly documented to play a critical role in animal models of seizures (6-13). These inhibitory receptors are pentamers formed by assembly of multiple subunit subtypes (α1-α6, β1-β3, γ1-γ3, δ, ε, π, θ, and ρ1-ρ3) that form chloride ion channels and most commonly contain two α subunits, two β subunits and a γ or δ subunit. $GABA_A$ receptors mediate both phasic, inhibitory synaptic transmission and tonic, perisynaptic inhibition, and several anti-epileptic drugs including benzodiazepines, barbiturates and neurosteroids act by enhancing $GABA_A$ receptor currents (14).

Mutations or variants in *GABRA1*, *GABRB3*, *GABRG2* and *GABRD* $GABA_A$ receptor subunit genes have been associated with multiple IGE syndromes (Figure 1). Most of these mutations have autosomal dominant inheritance and have been associated with epilepsy syndromes with pure febrile seizures (FS) (15), CAE (16), mixed afebrile and febrile seizures (CAE and FS and GEFS+ including Dravet syndrome) and afebrile seizures (4,17-21). These mutations include missense, nonsense and frame shift mutations in coding regions as well as mutations in noncoding regions.

The missense mutations or variants are in *GABRA1* (A322D) (22), *GABRB3* (P11S, S15F, G32R) (16), *GABRG2* (R43Q, R138G, K289M) (4,15,18) and *GABRD* (E177A, R220C and R220H) (20) subunit genes (Figure 1). In general, these mutations cause trafficking and/or channel gating defects (Table 1) (23-32). Mutations/polymorphisms in the *GABRB3* promoter have been shown to produce reduced binding of the neuron-specific the transcriptional activator N-Oct-3 and reduced gene transcription (33).

About one third of human genetic diseases are caused by nonsense mutations that generate PTCs that would result in production of a truncated protein and are often, but not always, associated with more severe genetic diseases than are associated with missense mutations. There are five PTC-generating mutations among sixteen mutations/variants/polymorphisms in $GABA_A$ receptor subunit genes that have been associated with genetic epilepsies (Figure 1). The PTC mutations include nonsense (*GABRG2*(Q1X) (34), *GABRG2*(Q351X) (17), *GABRG2*(W390X) (19)) mutations and a deletion/frameshift (*GABRA1*(S326fs328X) (5)) mutation. In addition, an intron splice donor site mutation, (*GABRG2*(IVS6 + 2T→G) (21)), has been predicted to cause skipping of exon 6 and creation of a PTC at the exon 5/exon 7 splice junction. Both *GABRG2*(Q1X) and *GABRG2*(Q351X) PTC mutations have been associated with Dravet syndrome, and the *GABRG2*(W390X) PTC mutation has been associated with FS and GEFS+. The *GABRA1*(del975C, S326fs328X) deletion/frameshift mutation has been associated with CAE in a singleton, and the *GABRG2*(IVS6 + 2T→G) splice donor site mutation has been associated with a small pedigree with CAE and FS.

Mutant mRNAs containing PTCs are subject to incomplete NMD

Recent studies have proposed a model of the pathogenesis of human genetic epilepsies associated with PTCs in $GABA_A$ receptor subunit genes (24,25). The studies suggest that the human epilepsy syndromes associated with PTCs are caused by a combination of degradation of unstable subunit mRNA and of unstable, truncated subunit protein (Figure 2)

(24,25), and that this model of pathogenesis may be extended to several reported genetic epilepsies and other inherited disorders. In cells, transcription of mutant genes produces mutant mRNAs and translation of mutant mRNAs produces mutant proteins. At the mRNA level, cellular mRNA surveillance mechanisms often degrade mutant PTC-containing mRNAs by NMD, which is a post-transcriptional, but translation-dependent, mRNA quality control mechanism. NMD recognizes and selectively degrades mRNAs that contain a PTC that is 50-55 nucleotides upstream from an exon-exon junction (35) or mRNAs with an aberrantly configured 3′untranslated region (UTR) (36). NMD is translation-dependent since translating ribosomes recognize PTCs during the pioneering round of translation. NMD is also splicing-dependent and requires an exon junction complex deposited during intron splicing at an exon-exon junction located at least 50-55 nucleotides downstream of the PTC. Exon junction complexes consist of mRNA decay factors including *Upf-1* (*rent1*), which is an RNA helicase and an essential factor for activation of NMD. NMD rids cells of most transcripts containing PTCs and reduces intracellular levels of potentially deleterious truncated proteins (37). Most nonsense transcripts are reduced by cellular mRNA surveillance processes, including NMD, to ~5-25% of wild-type levels (36).

Using minigene constructs that contain at least one intron and therefore support mRNA splicing and editing, we demonstrated that $GABA_A$ receptor α1(975C, S326fs328X) subunit mRNA, which should be subject to NMD by either the “50 nucleotide boundary rule” (38) or the “faux 3′-UTR model” (36), activated NMD (24). The PTC-containing mutant mRNA was not as stable as wild-type subunit mRNA and was reduced, but not eliminated. This degradation of mutant mRNA was consistent in heterologous HEK 293T and HeLa cells and rat cortical neurons in culture. However, the extent of mutant mRNA reduction was variable among different cell systems and with different transfection methods. NMD is mediated through a conservative mRNA decay factor Upf1, and the results suggested that the extent of NMD depended upon the relative abundance of the mutant mRNAs and the robustness of the endogenous mRNA decay machinery, including the NMD core components such as Upf factors in the specific cell types. This may imply that there is a variation of NMD efficiency among neurons in different regions of the CNS and during different developmental time windows that could contribute to regional and developmental differences in the pathophysiological effects of mutant PTC-containing $GABA_A$ receptor subunits.

The efficiency of NMD is correlated with phenotype severity in other diseases

Studies on other diseases indicate that the efficiency of NMD is correlated with phenotype severity. Activation of NMD can rid cells of most mRNAs containing PTCs and reduce synthesis of truncated proteins that have potentially deleterious effects inside cells. This may reduce the manifestation of some genetic diseases if the wild-type allele is haplosufficient for physiological function or may only produce a mild phenotype compared to some C-terminal truncation mutations that have dominant-negative effects on wild-type proteins. Triggering NMD and escaping NMD may cause distinct disease phenotypes (39). In osteogenesis imperfecta, Stickler syndrome and Marfan’s syndrome, it has been postulated that NMD moderates the phenotype compared with that produced by missense mutations. Marfan’s syndrome is an autosomal dominant systemic disorder of connective tissue caused by mutations in the extracellular matrix protein fibrillin 1 (FBN1) (40). Fibrillin is the major constitutive element of extracellular microfibrils and has a widespread distribution in both elastic and nonelastic connective tissue throughout the body. A genotype-phenotype difference has been noticed in Marfan’s syndrome; patients with low levels of mutant fibrillin 1 due to NMD often exhibit milder phenotypes that fall outside of the clinical criteria required for a Marfan’s syndrome diagnosis. It was suggested that the low levels of mutant transcript (about 7-10% of wild-type levels) may produce sufficient amounts of

truncated fibrillin 1 to produce dominant-negative interference with the wild-type allele, leading to severe Marfan's syndrome (41).

A molecular mechanism similar to that in Marfan's syndrome may help explain why mutations in the same gene cause different clinical phenotypes and with different disease severity within $GABA_A$ receptor gene mutations associated with IGEs. Namely, how complete NMD is in an individual may be associated with the intrafamilial phenotypic variation among family members carrying the same mutation. Similar discrepancies in genotype-phenotype correlation have been reported for $GABA_A$ receptor γ2(+/−) knock-out mice that displayed only a hyperanxiety phenotype, and in humans who have an early exon PTC-causing splice-donor site mutation that was associated with CAE and febrile seizures (21) and a C-terminal γ2 subunit truncation, Q351X, that was associated with the more severe GEFS+ syndrome (17). The pathogenesis of the GABRA1(975delC, S326fs328X) deletion/frame shift mutation is due mainly to NMD with a small portion of mutant transcripts escaping NMD and generating a truncated mutant protein that is degraded by ERAD. The mutant α1(S326fs328X) subunit protein had no dominant negative effect on the wild-type channel function when coexpressed with the wild-type subunits.

Truncated mutant proteins are subject to ERAD, but with different rates

Since NMD 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 and/or ERAD after translation. Previous studies (16,23,24,32) have demonstrated that truncated mutant proteins translated from mutant mRNAs that escape NMD are often trafficking deficient, misfolded and misrouted and consequently are subject to ERAD as are other non ion channel proteins (42). The cellular fate of mutant truncated $GABA_A$ receptor subunit proteins may be similar to that of misfolded and trafficking deficient missense mutant subunits (23,43). The mechanisms by which ERAD targets misfolded proteins includes the ubiquitin-proteasomal system (44) and the autophagy/lysosome pathway (45). 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. In addition, since the efficiency of NMD may be variable, there may be a different balance of reduction of mutant mRNA by NMD or reduction of mutant protein by ERAD in neurons in different regions of the brain, during different developmental stages or among different individuals.

Subunit truncations produce loss of function alleles that are often trafficking-deficient

Our studies on two different PTC-generating mutations in different $GABA_A$ receptor subunit genes demonstrated that both mutations caused a loss of subunit function. When expressed with partnering subunits in HEK 293T cells, mutant subunits had minimal expression on the cell surface compared with wild-type subunits. However, surface expression of the mutant subunits was not totally absent. Using whole cell recording from wild-type and mutant α1β2γ2S receptors, we demonstrated that very small, often nondesensitizing currents were recorded from HEK 293T cells coexpressing either α1(975delC, S326fs328X)β2γ2S or α1β2γ2S(Q351X) subunits. The currents were significantly smaller than with coexpression of the missense mutation-containing γ2S(R43Q) subunit with α1β2 subunits, although the missense mutation caused a trafficking defect in the subunits. The nature of the small currents with expression of the truncated mutant subunits is not clear but may represent misfolded homomers or multimers of the

mutant and/or wild-type subunits. However, it is not clear if these misfolded homomers or multimers observed in our *in vitro* cellular system would be present on the cell surface and in synapses of neurons nor is their functional and cellular fate clear since most of our observations were made in an acute overexpression cell systems. Nevertheless, the amount of truncated mutant subunits on the surface and synapses would be minimal based on our study on α1 and γ2 subunit truncation mutations.

Truncated $GABA_A$ receptor subunits are immature and subject to ERAD

Like all glycoproteins, $GABA_A$ receptor subunits are subject to ER quality control. Only correctly folded, assembled and mature subunits successfully oligomerize with other subunits and present on the surface and in synapses as pentamers (Figure 2). Previous work has demonstrated that cell surface $GABA_A$ receptor subunits are more mature and have a higher molecular mass compared with immature subunits inside the ER (22,23,45). Our studies have demonstrated that both α1(975delC, S326fs328X) and γ2S(Q351X) subunits that were transcribed from mutant mRNA that escaped NMD were retained in the ER and were subject to glycosylation arrest (22,23). When coexpressed with partnering β2 and γ2 subunits, α1(975delC, S326fs328X) subunits displayed only core, ER glycosylation and when coexpressed with partnering α1 and β2, γ2S(Q351X) subunits also displayed only core glycosylation (23). The glucosidase Endo-H removes high-mannose N-linked carbohydrates attached in the ER but not those attached in the trans-Golgi region. In contrast the glucosidase PNGaseF removes carbohydrates attached in both ER and trans-Golgi regions. On western blot of HEK 293T whole cell lysates, Endo-H digestion shifted the α1(975delC, S326fs328X) and γ2S(Q351X) subunit protein main bands to the same levels obtained with PNGaseF digestion, suggesting that when coexpressed with partnering subunits both α1(975delC, S326fs328X) and γ2S(Q351X) subunit protein underwent ER, but not Golgi, glycosylation. When immature subunits attain only core glycosylation, they are not trafficked to the cell surface or to synapses and are eventually degraded. In the heterozygous disease condition, wild-type subunits that are correctly folded and assembled will be transported in vesicles from the ER to the trans-Golgi and then further trafficked to the cell surface and synapses with associated proteins like GABARAP, gephyrin and PRIP. PRIP has recently been identified as a molecule that binds to GABARAP and plays an important role in receptor signaling, particularly in GABARAP- and phosphorylation-dependent $GABA_A$ receptor modulation (46). Surface receptors are then endocytosed and recycled or degraded by lysosomes. In contrast, misfolded, un or mis-assembled or misrouted mutant subunits, and in some conditions wild-type subunits, will be retained in the ER and subsequently degraded through lysosome or ubiquitin-proteasome pathways (Figure 2).

There are two categories of truncated $GABA_A$ receptor subunit proteins

Based on our work, the two truncated, mutant $GABA_A$ receptor α1(975delC, S326fs328X) and γ2S(Q351X) subunits had different effects on wild-type receptor channel functions. In cell systems *in vitro*, we co-expressed wild-type and mutant subunits to mimic the patient's autosomal dominant inheritance in which wild-type and mutant alleles coexist.

With mixed expression, mutant α1(975delC, S326fs328X) subunits had minimal or no negative effect on wild-type α1 subunit biogenesis, trafficking or function

The PTC-generating α1 subunit deletion/frameshift mutation, 975delC, S326fs328X, is associated with a mild epilepsy phenotype, CAE. In cell systems *in vitro*, using NMD sensitive minigenes and a half minigene dose as control for the gene deletion condition (α1(+/−)β2γ2S receptors), peak current amplitudes recorded from cells expressing mixed α1/α1(S326fs328X)β2γ2S receptors were not significantly different than current amplitudes recorded from α1(+/−)β2γ2S receptors. Surface α1 subunit expression in the mixed

condition was also similar to that produced by the half minigene dose condition. With mixed coexpression of wild-type α1 and mutant α1(975delC, S326fs328X) subunits, wild-type subunits were abundantly present while mutant α1(975delC, S326fs328X) subunit levels were minimal. Thus we speculate that the epilepsy manifested in the patients harboring this type of mutation may result primarily from *GABRA1* functional haploinsufficiency. Channel function produced in the heterozygous condition may be similar to that produced by the hemizygous condition.

With mixed expression, mutant γ2(Q351X) subunits had a dominant negative effect on wild-type receptor biogenesis, trafficking and function

The proband of the PTC-generating γ2 subunit mutation, Q351X, had a severe type of epilepsy, Dravet syndrome. In contrast to α1(975delC, S326fs328X) subunits, biochemical and functional studies indicated that mutant γ2(Q351X) subunits were likely associated with a more severe pathophysiology. Using a half minigene dose as a control for the gene deletion condition, peak current amplitudes recorded from mixed α1β2γ2/γ2S(Q351X) receptors was significantly smaller than that recorded from α1β2γ2S(+/−) receptors. With mixed γ2S/γ2S(Q351X) subunit expression, surface γ2S subunits and partnering α1 and β2 subunits were also reduced compared to that produced by the half minigene dose condition. We had demonstrated that in the presence of mutant γ2(Q351X) subunits, both surface and total α1 subunit levels were reduced due to premature degradation (25). Thus, we speculate that the epilepsy manifested in the patients harboring this type of mutation may result from haploinsufficiency plus a dominant negative effect of the mutant subunit on the wild-type receptor channels. $GABA_A$ receptor channel function produced in the heterozygous condition may be less than that produced in the hemizygous condition.

$GABA_A$ receptor profiles are altered when either γ2 or α1 subunits are reduced by PTCs

Our data on hemizygous control receptors and studies of the heterozygous γ2 subunit knock-out mouse (47) both suggested that α1β2 receptors are formed and trafficked to the surface when there is a reduced expression of γ2 subunits. This suggests that α1β2 receptors will be formed if γ2 subunits are reduced or unavailable, resulting in a small compensation for the loss of $GABA_A$ergic inhibition. In the presence of mutant γ2(Q351X) subunits, α1β2 receptors were formed at a reduced level compared to that obtained with expression of α1 and β2 subunits alone and α1β2 receptor levels were progressively reduced by overexpression of mutant γ2(Q351X) subunits. In patients harboring this mutation, there may be α1β2 receptors coexisting with α1β2γ2 receptors. However, the presence of α1β2 receptors may depend on the relative expression of each subunit. This also implies that in these patients there would be altered channel kinetics due to the alteration of subunit composition of surface receptors and that there might be different responses to antiepileptic drugs, since α1β2 and α1β2γ2 receptors have different pharmacological properties. In addition, we speculate that when α1 subunit levels are reduced by PTC mutations, there will be an increased expression of other α subunits such as α3 and α2 subunits that may vary in different brain regions (48,49). In summary, reduction of either total functional γ2 or α1 subunits by PTC mutations would alter the $GABA_A$ receptor profiles in brain.

There is a destructive interaction between wild-type and mutant γ2(Q351X) subunits

The presence of mutant γ2(Q351X) subunits produced a dominant negative suppression of the biogenesis of wild-type γ2 subunits. It is likely that some other truncation mutations will have a similar dominant negative effect. Biochemical data indicated that the dominant

negative effect of mutant subunits on wild-type subunits was likely due to oligomerization of mutant and wild-type subunits, resulting in ER retention and glycosylation arrest of both wild-type and mutant subunits. Retained, immature wild-type subunits were rapidly degraded by ERAD through the ubiquitin-proteasome system. As a result, the γ2 subunit mutation, Q351X, reduced mature surface $GABA_A$ receptors to an extent that was greater than that resulting from hemizygous expression of the γ2 subunit. This combination of loss of surface expression and function of mutant subunits and the dominant negative effect of mutant subunits on wild-type subunits would result in a considerable loss of inhibition and is the likely explanation for the pathogenesis of GEFS+ including Dravet syndrome associated with this mutation. Thus we speculate that the epilepsy manifested in patients harboring this type of mutation may result from loss of function of one allele and a dominant negative suppression of wild-type $GABA_A$ receptors.

The mutant γ2(Q351X) subunit protein has a “gain of toxic function” in addition to the loss of the function of the mutant allele itself. This effect could be due to direct interactions of mutant and wild-type subunits as suggested above and also due partially to the mutant protein triggering ER stress or the unfolded protein response. The unfolded protein response reduces the burden on the ER resulting from unfolded or trafficking deficient molecules through a series of cellular processes including inhibition of transcription, rescue of unfolded protein by increasing the expression of chaperones and ERAD (50,51). Future study focusing on transcription, recycling and degradation may further elucidate the underlying mechanisms of this trafficking deficient mutant in epileptogenesis.

GABRA1(+/−) and GABRG2(+/−) mice do not adequately mirror loss of function epilepsy mutations

It is well established that impairment of $GABA_A$ receptor function leads to epilepsy, and thus $GABA_A$ receptor subunit gene deletion mice have been created to study epilepsy. However, these heterozygous gene deletion knock-out animals do not adequately mirror human loss of function epilepsy mutations. For example, homozygous *GABRA1* gene deletion knockout mice only manifest tremor while heterozygous mice are behaviorally normal (52). In contrast, the human *GABRA1* gene mutation, 975delC, S326fs328X, should create a loss of function allele based on studies *in vitro* but was associated with CAE in humans. Likewise, heterozygous *GABRG2* gene deletion knockout mice only manifest hyperanxiety and are seizure-free (53). In contrast, the human *GABRG2* gene mutation, Q351X, should create a loss of function allele based on the studies in *vitro*, but was associated with Dravet syndrome in the proband of the pedigree. The molecular mechanisms underpinning the discrepancy between the gene deletion knock-outs of α1 and γ2 subunit genes are still unclear and may in part be due to species variation or genetic background. However, our recent work suggests that gene deletion knock-outs and the presence of a loss of function allele may also have different pathophysiological effects. The functional and cellular consequences from a mutation resulting in a loss of function allele may be fundamentally different than simple deletion of an allele, since molecular events such as NMD, ERAD and the unfolded protein response are not activated in heterozygous gene deletion animals.

Therapeutic implications

These recent studies of $GABA_A$ receptor epilepsy mutations provide molecular targets for potential new therapeutic strategies for treatment of genetic epilepsies. Potential therapeutic approaches would include increasing wild-type and/or mutant $GABA_A$ receptor channel function or decreasing the disturbance of the cellular signaling by the presence of the mutant $GABA_A$ receptor subunit protein. For those $GABA_A$ receptor subunit mRNAs with PTCs

that activate NMD and cause epilepsy due to loss of function of one allele, increasing translational read-through and generating a full-length protein may increase channel function. In addition, this approach would reduce non-functional, truncated protein that may have cellular toxicity. Promotion of in-frame read-through of PTCs may hold great promise due to current drug availability. There are two types of drug that are used to promote read-through: aminoglycosides and PTC124. Aminoglycosides are established antibiotics that have been shown to affect the ribosome decoding site and to promote read-through, but they have significant side effects when administered at high doses or with prolonged dosing including hearing loss and kidney damage (54). There are a number of aminoglycosides that have been used to promote read-through but their efficiency is variable (54). Promotion of read-through with aminoglycoside treatment has already been used in patients with several different diseases including cystic fibrosis and Duchenne muscular dystrophy (54,55), in addition to numerous studies with other diseases (Table 2) (56-74). PTC124 is a new, orally bioavailable agent that selectively induces ribosomal read-through of premature, but not normal, translation-termination codons. It is a small organic molecule with no antibiotic properties. Unlike aminoglycosides, PTC124 has no serious side effects (75). Clinical trials of PTC124 have been performed in Duchenne muscular dystrophy and cystic fibrosis, and PTC124 was well tolerated and promoted dystrophin production in primary muscle cells in humans and MDX mice (65). Several ongoing PTC124 clinical trials will provide more valuable information about this therapy. The same strategy with PTC124 may be useful for some patients with epilepsy who harbor PTC-generating mutations if the mutant protein is otherwise normal and functional. However, work in this area has to proceed with caution. For example it is imperative to demonstrate that the mutant read-through protein is functional, has no dominant negative effects and does not activate the unfolded protein response. In summary, recent studies suggest that the pathology of the PTC-generating mutations is likely to be due to a combination of reduction of channel function and disturbance of cellular homeostasis due to the presence of small amounts of mutant protein. Thus, a therapeutic strategy to eliminate production of mutant protein using siRNA targeting of the mutant transcripts might also be a useful approach (76).

Concluding remarks

The recent research developments on $GABA_A$ receptor subunit nonsense mutations suggest potential novel therapeutic approaches for genetic epilepsies. Increasing production of functional full-length protein or attenuating production of nonfunctional truncated protein will likely be the most useful therapeutic approaches in addition to using conventional antiepileptic drugs. As described above, recent studies suggest that ion channel PTC mutations can cause NMD, and that neurons are likely to share common mRNA surveillance mechanisms present in other cell types, thus extending our understanding of the molecular underpinning of these epilepsies and providing new molecular targets for developing new therapeutic strategies to treat ion channel diseases. It is likely that both NMD and ERAD molecular pathways contribute to genetic epilepsies caused by PTC-generating mutations (Figure 2). The reduced channel function is due to loss of function of the mutant allele with or without dominant negative suppression of partnering wild-type subunits depending on the type of mutation. We speculate that simple loss of one allele in $GABA_A$ receptor $\alpha 1$ or $\gamma 2$ subunit genes may not be sufficient to cause seizures, although seizure threshold will be lowered due to the lack of one allele. However, in loss of function mutation harboring patients, there may be additional mechanisms that are related to the intracellular processing of mutant subunits. Based on these recent research developments, promotion of translational read-through and elimination of nonfunctional truncated protein are likely to be the potential therapeutic approaches.

Box 1

Genetic epilepsy: the clinical picture

Genetic epilepsies refer to epilepsies caused by gene mutations. These epilepsies are virtually synonymous with idiopathic epilepsies, which are usually self-limiting and are not associated with neurological abnormalities. Genetic epilepsies are often, but not always, associated with mutations in voltage-gated ion channels like sodium, potassium and calcium channels or ligand-gated ion channels like $GABA_A$ receptor and nicotinic acetylcholine receptor subunit gene mutations. Diagnosis of these epilepsies is largely based on seizure history, family history, characteristic clinical seizure patterns and electroencephalographic findings. Genetic advances identified some genotype-phenotype correlations. For example, febrile seizures and febrile seizure with generalized epilepsy plus have been frequently associated with sodium channel *SCN1A*, *SCN1B* and $GABA_A$ receptor *GABRG2* mutations; childhood absence epilepsy has been frequently associated with *GABRB3* and *GABRG2* mutations. The more severe epilepsy syndrome, Dravet syndrome, has been associated with truncation mutations in *SCN1A* and *GABRG2*. Most studies characterizing the molecular pathology of these mutations have been *in vitro*. Since there is no specific treatment available, clinical challenges remain to develop individualized therapeutic regiments for these patients.

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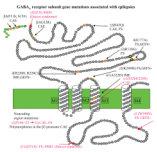

Figure 1. Schematic representation of a $GABA_A$ receptor subunit topology, showing the location of epilepsy mutations identified by different groups up to date

The red color was used for PTC generating mutations. Note that to be consistent with previous publications, mutation locations in the γ2 subunit are numbered from the start of the mature peptide while mutation locations in the α1, β3 and δ subunits are numbered from the start of the precursor protein that includes the signal peptide. The γ2 subunit mutation, R139G, was renumbered as R138G due to a counting error in a previous publication. Abbreviations: CAE, childhood absence epilepsy; FS, febrile seizures; GEFS+, generalized epilepsy with febrile seizures plus; JME, juvenile myoclonic epilepsy

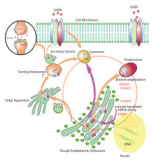

Figure 2. Schematic representation showing the $GABA_A$ receptor subunit biogenesis, assembly and trafficking

PTC-generating mutant subunits (mutant) are subject to NMD and ERAD. Therefore, the truncated mutant subunits are unlikely to be present on the cell surface and in synapses as are wild-type receptors. Many PTC-generating mutant mRNAs are subject to NMD. The mRNAs escaping from NMD will be translated and subject to ERAD due to the trafficking deficiency of the translated mutant subunits. The arrows designate the targeted subcellular locations of wild-type or mutant subunits. The circle represents the subcellular locations to which the mutant PTC-generating $GABA_A$ receptor subunits are likely trafficked.

Table 1

$GABA_A$ receptor subunit mutations/variants and polymorphisms associated with genetic epilepsies and their postulated molecular defects

gene	mutations/variants/ polymorphisms	locus	postulated mechanisms	phenotypes	references
GABRA1	A322D mutation	5q34	ERAD	JME	22,26
GABRA1	975delC, S326fs328X mutation	5q34	NMD, ERAD	CAE	5,24
GABRB3	−897 T/C polymorphism	15q11-14	decreased transcription	CAE	33
GABRB3	P11S mutation	15q11-14	hyperglycosylation?	CAE	16
GABRB3	S15F mutation	15q11-14	hyperglycosylation?	CAE	16
GABRB3	G32R mutation	15q11-14	hyperglycosylation?	CAE	16
GABRG2	Q1X (Q40X) mutation	5q34	unclear	Dravet	34
GABRG2	R43Q (R82Q) mutation	5q34	Impaired oligomerization, ER Retention	CAE,FS	4,23,27,28,32
GABRG2	R138G (R177G) mutation	5q34	unclear	FS	15
GABRG2	K289M (K328M) mutation	5q34	gating defect	FS, GEFS+	18,28,29
GABRG2	Q351X (Q390X) mutation	5q34	ER Retention, ERAD, dominant negative effect	FS, Dravet	17,25
GABRG2	IVS6+2T-G mutation	5q34	exon skipping?	CAE, FS	21
GABRG2	W390X (W429X) mutation	5q34	unclear	FS, GEFS+	19
GABRD	E177A mutation	1p36	gating defect, impaired trafficking?	GEFS+	20,30
GABRD	R220C mutation	1p36	unclear	GEFS+	20
GABRD	R220H variant	1p36	gating defect, impaired trafficking?	JME	20,30

Abbreviations: NMD, nonsense-mediated mRNA decay; ER, endoplasmic reticulum; ERAD, ER associated degradation; CAE, childhood absence epilepsy; FS, febrile seizures; GEFS+, generalized epilepsy with febrile seizures plus; JME, juvenile myoclonic epilepsy

Table 2

Studies of other diseases caused by PTCs that responded to read-through promoting drugs

Gene	Diseases	Read-through drug	System	References
ATM	Ataxia-telangiectsia	aminoglycosides	*in vitro*	56
ATP2C1	Hailey-Hailey disease	aminoglycosides	*in vivo*	57
AVPR2	X-linked nephrogenic diabetes insipidus	aminoglycosides	*in vitro*	58
CFTR	cystic fibrosis	aminoglycosides	*in vitro/vivo*	59,60
CFTR	cystic fibrosis	PTC124	*in vivo*	61
CTNS	Nephropathic cystinosis	aminoglycosides	*in vitro*	62
DMD	Duchenne Muscular Dystrophy	aminoglycosides	*in vitro/vivo*	63,64
DMD	Duchenne Muscular Dystrophy	PTC124	*in vitro*	65
FVII	Factor VII deficiency	aminoglycosides	*in vitro*	66
FVIII	hemophilia A	aminoglycosides	*in vivo*	67
FIX	Hemophilia B	aminoglycosides	*in vivo*	68
KCNA5	Familial atrial fibrillation	aminoglycosides	*in vitro*	69
PKD2	Polycystic kidney disease	aminoglycosides	*in vitro*	70
IDUA	Hurler syndrome	aminoglycosides	*in vitro*	71
RP2	X-linked retinitis pigmentosa	aminoglycosides	in *vitro*	54
SMN1	Spinal muscular atrophy	aminoglycosides	*in vitro*	72
SMN2	Spinal muscular atrophy	aminoglycosides	*in vitro*	73
PCDH15	Usher syndrome	aminoglycosides	*in vitro*	74