1 $GABA_A$ RECEPTORS

The γ-aminobutyric acid (GABA) type A receptors ($GABA_A$ receptors) are ligand-gated chloride (Cl^-) channels that mediate major inhibitory functions in the central nervous system (CNS). Many antiepileptic drugs (AEDs) are $GABA_A$ receptor agonists, whereas $GABA_A$ receptor antagonists can be strong convulsants. Hence, $GABA_A$ receptors have been postulated to play a key role in the pathogenesis of epilepsy. In fact, mutations and variations in the genes encoding $GABA_A$ receptor subunits have been associated with certain types of epilepsy.

The GABA receptors include the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors. The $GABA_A$ receptors belong to the Cys-loop superfamily, which also includes acetylcholine, glycine, and serotonin 5-HT3 receptors. The subunits of this superfamily have four transmembrane domains, with the second transmembrane domain forming a central ion pore with its counterpart subunits (Macdonald and Olsen, 1994) (Figs. 1 and 2). When GABA, the physiological ligand of GABA receptors, binds to the receptor, the receptor's ion pore opens and allows Cl^- to pass through the cell membrane.

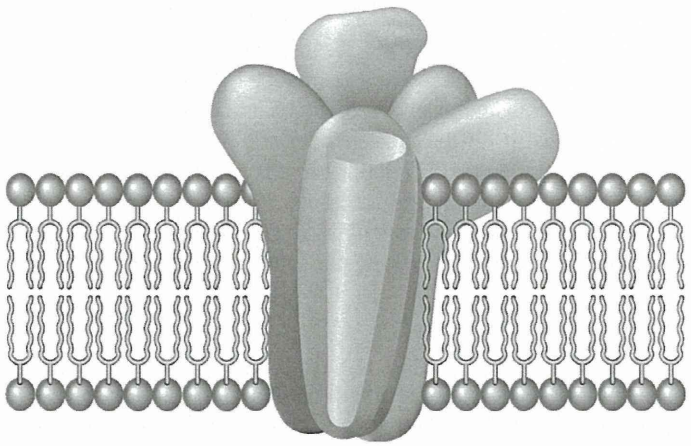

FIGURE 1

Structure of the $GABA_A$ receptor. $GABA_A$ receptors, serving as the main inhibitory component in neuronal networks, are a ligand-gated ion channels comprising two α, two β subunits, and one γ or δ subunit making it is a heteropentamer. There are other subunits, such as θ, π, ρ, and each subunit has subtypes (e.g., α1, α2), which make the configuration of the $GABA_A$ receptor very heterogeneous. It is believed that the main subtypes of α, β, and γ subunits in human brain are α1, β2, and γ2 subunits, which are encoded in the independent genes, *GABRA1*, *GABRB2*, and *GABRG2*, respectively. Although receptors consisting of only α and β subunits exhibit ion channel function *in vitro*, the γ subunit has diazepam binding sites and is considered to play an important role in receptor function *in vivo*. Several complex mechanisms are involved in the proper assembly and sorting of $GABA_A$ receptor. Mutations known to be associated with human epilepsies have been the identified in the genes encoding α1, α6, β2, β3, γ2, and δ subunits.

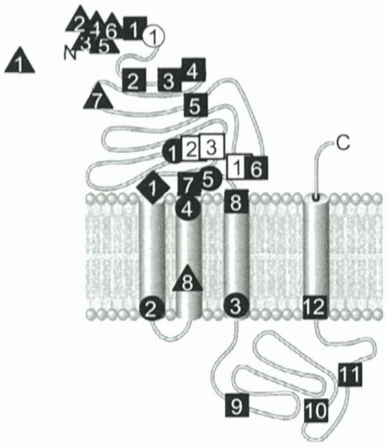

FIGURE 2

Genetic abnormalities or variations in $GABA_A$ receptor subunits. Each subunit, α, β, γ, δ, θ, π and ρ subunit, has a long N-terminus and four membrane spanning domains (TM1–4) as well as a long loop between TM3–4. The N-terminus is considered crucial for subunit assembly. In the γ subunit, there are benzodiazepine binding sites at the N-terminus. TM2 lines the ion pore of the receptors with the corresponding part of counterpart subunits. Thus, incorporation of the γ subunit renders the receptor benzodiazepine-sensitive (Zhu et al., 2008). ● indicates mutations of *GABRA1*, the gene encoding α1 subunit; ○ indicates a mutation of *GABRA6*, the gene encoding the α6 subunit; ◆ indicates a mutation of *GABRB2*, the gene encoding the β2 subunit; ▲ indicates mutations of *GABRB3*, the gene encoding the β3 subunit; ■ indicates mutations of *GABRG2*, the gene encoding the γ2 subunit; and □ indicates mutations or variant of *GABRD*, the gene encoding the δ subunit. The numbers in the symbols correspond to the numbers of the mutations listed in Table 1.

$GABA_A$ receptors function predominantly as heteropentamers and contain five of the following eight subunit types: α, β, γ, δ, ε, π, θ, and ρ (Macdonald and Olsen, 1994). In total, there are 19 subtypes of these 8 subunits, including α1–6, β1–3, γ1–3, δ, ε, π, θ, and ρ1–3. The most abundant $GABA_A$ receptors in the brain are thought to contain two α, two β, and one γ or δ subunit (Baumann et al., 2002) (Fig. 1). Binary receptors containing only α and β subunits have channel functions and β3 can form a homopentamer. However, these receptors exist in small numbers under normal physiological conditions.

In the adult brain, the $GABA_A$ receptor serves as the primary inhibitory component in neural networks that mediate phasic inhibitory synaptic or tonic and perisynaptic inhibitory transmission. Phasic inhibition is mediated chiefly by the $GABA_A$ receptor isoform that contains αβγ subunits, whereas tonic inhibition is mediated by the isoform that contains αβδ subunits (Bai et al., 2001; Saxena and Macdonald, 1994; Stell et al., 2003; Wei et al., 2003). The $GABA_A$ receptor produces its inhibitory actions by allowing a Cl^- influx through its pore, which

hyperpolarizes the membrane. Once the pore opens, the direction of the Cl^- current, that is, whether the current is an influx or efflux, depends on the intracellular concentration of Cl^-, which is regulated by the chloride–potassium (KCC2) and chloride–potassium–sodium (NKCC1) cotransporters. In the mature brain, KCC2 decreases the intracellular Cl^- concentration; thus, opening the $GABA_A$ receptor pore will produce a Cl^- influx current that hyperpolarizes the cell membrane. In developing brains in which KCC2 has not yet developed, NKCC1 acts to increase intracellular Cl^- concentrations, and as a result, there is an efflux of Cl^- through the $GABA_A$ receptor. This efflux, however, depolarizes the cell membrane; thus, the actions of the $GABA_A$ receptor in the immature brain are excitatory (Fukuda, 2005).

Many drugs that act on the CNS target $GABA_A$ receptors, such as sedatives, anxiolytics, and AEDs. In addition to these drugs, endogenous and exogenous substances such as neurosteroids and ethyl alcohol exert their effects on the CNS via $GABA_A$ receptors. Hence, these drugs and substances amplify Cl^- influx, which is generated by binding of GABA. This phenomenon is called potentiation. Many AEDs, such as barbiturates, topiramate, and benzodiazepines, are $GABA_A$ receptor agonists. Among these AEDs, the actions of benzodiazepines on $GABA_A$ receptors are the most clinically relevant and best characterized. Benzodiazepines require the γ subunit to potentiate $GABA_A$ receptor functions. In contrast, $GABA_A$ receptor antagonists can be strong convulsants, such as picrotoxin, a neurotoxin.

Dysfunctions of $GABA_A$ receptors have been postulated to underlie the pathogenesis of epilepsy. In fact, mice genetically engineered with deficiencies in several $GABA_A$ receptor subunits show seizure phenotypes. $GABA_A$ receptors have been directly implicated in the pathomechanisms of human epilepsy in that mutant $GABA_A$ receptor subunits have been identified in idiopathic epilepsy.

2 MUTATIONS AND GENETIC VARIATIONS OF THE $GABA_A$ RECEPTOR

Mutations and genetic variations of $GABA_A$ receptors have been associated with epilepsy (Table 1). However, only a limited number of epilepsy types result from such mutations, which are not necessarily the direct cause of epilepsy (Kim et al., 2012; Ma et al., 2006a,b; Xiumin et al., 2007). Nevertheless, the list of $GABA_A$ receptor mutations associated with epilepsy is expected to grow in the future. Furthermore, these $GABA_A$ receptor mutations have revealed new aspects of the pathomechanisms of epilepsy.

In this chapter, the amino acids of the $GABA_A$ receptor subunits are named according to the latest recommendations for the nomenclature by Human Genomic Variation society (http://www.hgvs.org/mutnomen/) (den Dunnen and Antonarakis, 2000). The numbering beginning with the first methionine of nascent proteins (i.e., including the signal peptides of the protein) may differ from that seen in their original descriptions, especially for mutations of the $\gamma 2$ subunit.

Table 1 $GABA_A$ receptor subunit mutations/variations associated with epilepsies

•α1 subunit (5q34) GABRA1			
1	D219N	GGE	Lachance-Touchette et al. (2011)
2	T292I	IS	Allen et al. (2013)
3	K353delins18*	GGE	Lachance-Touchette et al. (2011)
4	A322D	ADJME	Cossette et al. (2002)
5	S326fs2*	CAE	Maljevic et al. (2006)
○α6 subunit (5q31.1-q35) GABRA6			
1	R46W	CAE	Dibbens et al. (2009)
◆β2 subunit (4p13-p12) GABRB2			
1	F246S	IS	Allen et al. (2013)
▲β3 subunit (15q11-13) GABRB3			
1	−897T>C (polymorphism)	CAE	Urak et al. (2006)
2	P11S (rare SNP)	CAE	Delahanty et al. (2011), Lachance-Touchette et al. (2010), Tanaka et al. (2008)
3	S15F	CAE	Tanaka et al. (2008)
4	N25D	IS	Allen et al. (2013)
5	G32R	CAE	Tanaka et al. (2008)
6	D35N	LGS	Allen et al. (2013)
7	E109G	LGS	Allen et al. (2013)
8	Y302C	LGS	Allen et al. (2013)
■γ2 subunit (5q34) GABRG2			
1	Q40*	Dravet	Ishii et al. (2014)
2	N79S	GGE	Shi et al. (2010)
3	R82Q	CAE, FS	Wallace et al. (2001)
4	P83S	GGE	Lachance-Touchette et al. (2011)
5	R136*	GEFS+	Johnston et al. (2014)
6	R177G	FS	Audenaert et al. (2006)
7	R323Q	GEFS+	Carvill et al. (2013)
8	K328M	FS, GEFS+	Baulac et al. (2001)
9	Q390*	FS, Dravet	Harkin et al. (2002)
10	IVS6+2T>G	CAE, FS	Kananura et al. (2002)
11	W429*	GEFS+	Sun et al. (2008)
12	Y444Mfs51*	GEFS+	Tian et al. (2013)
□δ subunit (1p36) GABRD			
1	E177A	GEFS+	Dibbens et al. (2004)
2	R220C	GEFS+	Dibbens et al. (2004)
3	R220H (variant)	JME	Dibbens et al. (2004)

GGE, genetic (idiopathic) generalized epilepsy; ISs, infantile spasms; ADJME, autosomal dominant juvenile myoclonic epilepsy; CAE, childhood absence epilepsy; LGS, Lennox–Gastaut syndrome; Dravet, Dravet syndrome; FSs, febrile seizures; GEFS+, genetic epilepsy with febrile seizures plus.

3 MUTATIONS OF THE α SUBUNIT

The α subunit is the requisite subunit for $GABA_A$ receptors, as it is the GABA binding site that initiates GABA-evoked potentials and forms the benzodiazepine binding site with the γ subunit. Several mutations of the genes encoding the α1 (*GABRA1*) and α6 (*GABRA6*) subunits have been identified in autosomal dominant juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), and infantile spasms (ISs).

3.1 MUTATIONS OF *GABRA1*

3.1.1 Mutations in Autosomal Dominant JME

A missense mutation, A322D of *GABRA1*, was identified in individuals with autosomal dominant JME (Cossette et al., 2002). Autosomal dominant JME is considered a unique subtype of JME because it has an autosomal dominant inheritance with high penetrance, which differs from common JME where inheritance is not obvious (Cossette et al., 2012). The A322D mutation is heterozygous and located in the third transmembrane domain, M3 (Fig. 1).

$GABA_A$ receptors reconstituted with α1 subunits that harbor the α1A322D mutation, along with β2 and γ2 subunits, show reduced GABA-evoked currents (Cossette et al., 2002). Subsequent experiments found that this reduction was due to reduced expression of the α1 subunit (Gallagher et al., 2004). Further studies showed retention of α1A322D improperly assembled with other subunits in the endoplasmic reticulum (ER). However, wild-type α1 subunits cotransfected with β2 and γ2 subunits underwent oligomerization, followed by normal expression on the plasma membrane (Gallagher et al., 2005). The apparent reduction of α1 subunit expression is thus attributed to the finding that α1A322D is depleted by trafficking defects, the degradation process in the ER, or endoplasmic reticulum-associated degradation (ERAD) (Meusser et al., 2005). ERAD is performed by the ubiquitin-proteasome system, which is initiated by the membrane insertion failure of the third transmembrane domain, M3, because the A322D mutation inhibits transmembrane helix formation (Gallagher et al., 2007). Furthermore, as a haploinsufficiency, α1A322D altered the time course of miniature inhibitory postsynaptic current (mIPSC) kinetics and reduced mIPSC amplitudes. An autosomal inheritance results from this haploinsufficiency, and the α1A322D retained in the ER alters the composition of wild-type $GABA_A$ receptors and exerts dominant-negative effects (Ding et al., 2010).

The majority of the mutations identified in $GABA_A$ receptor subunits are in the epilepsy phenotypes associated with febrile seizure (FS). However, autosomal dominant JME is not a FS-related epilepsy. This may be explained by the finding that the trafficking defect was followed by ERAD because the α1A322D is not affected by temperature, although the consequences of other mutated subunits are often aggravated by increased temperatures (Kang et al., 2006).

3.1.2 Mutations in Genetic (Idiopathic) Generalized Epilepsy

Three *GABRA1* mutations were identified in genetic (idiopathic) generalized epilepsy (GGE). One was identified in a single case of CAE, whereas the other two were identified in two nonrelated pedigrees where the affected individuals with the GGE phenotype presented with generalized tonic–clonic seizures (GTCSs) (Lachance-Touchette et al., 2011).

A *de novo* heterozygous *GABRA1* mutation, S326fs328*, was identified in a sporadic case of typical CAE without FS. The parentage was confirmed and the mutation was not present in the parents, younger brother, or 290 ethnically matched healthy individuals (Maljevic et al., 2006). $GABA_A$ receptors reconstituted with α1 subunits that harbor the α1S326fs328* mutation, along with β2 and γ2 subunits, showed null GABA-evoked currents that resulted from trafficking abnormalities followed by degradation of the mutant receptors (Maljevic et al., 2006). More meticulous investigations clearly showed that most mRNA of the *GABRA1* mutation (Kang et al., 2009b), S326fs328*, underwent nonsense-mediated mRNA decay (NMD), and any α1S326fs328* escaping from NMD was subjected to ERAD. This reduces the inhibitory transmission mediated by $GABA_A$ receptors, which causes epilepsy (Kang et al., 2009b).

A heterozygous mutation of *GABRA1*, K353delins18*, was identified in a family in which the affected individuals exhibited late-onset, afebrile, GTCSs, and photosensitivity whereas there was an obligate carrier (Lachance-Touchette et al., 2011). K353delins18* is a 25-bp insertional mutation that results in deletion of the fourth transmembrane domain and insertion of an aberrant 18-mer amino acid followed by a premature stop codon. A functional analysis of $GABA_A$ receptors reconstituted with mutated α1 subunits (α1K353delins18*) and wild-type β2 and γ2 subunits indicated these receptors exhibit no channel functions with GABA binding. Studies that investigated the subcellular localization of the $GABA_A$ receptors harboring the α1K353delins18* mutation indicated that the mutant $GABA_A$ receptors were not transported to the cell surface, but were retained in the ER. This trafficking abnormality is similar to that observed with α1A322D. Thus, the null channel function of the mutant $GABA_A$ receptors is thought to result from improper intracellular receptor trafficking, albeit the fate of the retained receptors is yet to be elucidated (Lachance-Touchette et al., 2011).

A heterozygous missense mutation of *GABRA1*, D219N, was also identified in a pedigree in which the affected individuals presented primarily with FS, with or without generalized tonic–clonic and absence seizures.

3.1.3 Mutations in IS

A *de novo* mutation was found in a massive sequencing study on a large cohort of epileptic encephalopathies. The mutation was identified as a heterozygous missense mutation, T292I, in a boy who presented with the IS phenotype and an initial FS at the age of 1 month; his subsequent seizures included IS, atonic, tonic, and atypical absence types. The patient showed significant developmental delay, but his MRI

findings were normal. His EEG showed generalized spikes and waves at 2.5 Hz and multiple foci of epileptiform activity. The functional study on the mutated $GABA_A$ receptor has yet to be performed.

3.2 MUTATIONS OF *GABRA6*

A mutation of *GABRA6* was identified in a screening for $GABA_A$ receptor subunit mutations in a large cohort consisting of genetic epilepsy with febrile seizures plus (GEFS+), GGE, and FS. The mutation was R46W of *GABRA6*, a heterozygous mutation identified in a patient with CAE and atonic seizures. This mutation was not identified in 96 control individuals (Dibbens et al., 2009).

3.2.1 Mutations in CAE

A functional study was conducted to investigate the consequence of the R46W mutation with $GABA_A$ receptors reconstituted in $\alpha6\beta2\gamma2$ or $\alpha6\beta2\delta$ configuration (Hernandez et al., 2011). Several aberrant ion channel properties were identified in the $GABA_A$ receptors harboring the mutated $\alpha6$ (R46W) in both subunit configurations. In brief, the gating properties of these mutated $GABA_A$ receptors were impaired. A reduced current density was present with both configurations, and the reduction was prominent in $\alpha6$(R46W)$\beta2\delta$. In addition, the desensitization increased in $\alpha6$(R46W)$\beta2\gamma2$ and its deactivation was slow. Single-channel analyses revealed the mean open time and burst duration of the mutant $GABA_A$ receptors was reduced. The reduced current density of the mutant $GABA_A$ receptors was attributed not only to the reduction of the open state in individual $GABA_A$ receptors but also to the reduction of the number of $GABA_A$ receptors on the cell surface. The reduced cell surface expression of $GABA_A$ receptors harboring the $\alpha6$(R46W) subunit resulted from improper assembly or trafficking defects. Moreover, a structural study revealed conformation changes consistent with the channel gating impairment. Thus, the $\alpha6$ (R46W) mutation causes impairment in both channel gating and cell surface expression of $GABA_A$ receptors.

3.2.2 Animals with Aberrant α Subunits

Mice lacking the $\alpha1$ subunit have been generated. The previously generated knockout mice with a mixed background strain did not present with seizure phenotypes, but anxiety-like behavior and tremors were observed (Kralic et al., 2002; Vicini et al., 2001). In a recent study, $\alpha1$ subunit-deficient mice were generated with a congenic strain; C57BL/6J exhibited a seizure phenotype resembling human CAE (Arain et al., 2012). Thus, whether an epilepsy phenotype develops depends on the genetic background. These findings are in agreement with the current notion that the majority of human idiopathic epilepsies are polygenic and result from multiple deficiencies in neural networks that could be created by the diversity of human genetic backgrounds.

GABAR6-deficient mice have been generated; however, an epileptic phenotype was not documented (Jones et al., 1997). $\alpha6$ disruption increased the degradation of

δ subunits, which mediates the $GABA_A$ receptor's tonic inhibition via peri- or extrasynaptic transmission. Thus, α6 subunit disruption is associated with δ subunit dysfunction. In granular cells of the mouse hippocampus, however, the loss of $GABA_A$ receptor tonic inhibition resulting from the combined loss of α6 and δ subunits may be alleviated by an increase in function for the two-pore-domain K^+ channel TASK-1, a voltage-independent K^+ conductance channel. This might explain why *GABAR6*-deficient mice showed no overt defects (Jones et al., 1997).

4 MUTATIONS OF THE β SUBUNIT

The β subunit is considered a major subunit of the $GABA_A$ receptor and is expressed predominantly in the human brain. The β2 subtype is considered the major constituent of the $GABA_A$ receptor in the adult brain, both quantitatively and spatially. In contrast, the β3 subtype is considered a major constituent in the developing brain, which indicates an important role in the developing brain, and hence in the etiology of childhood CNS disorders (Brooks-Kayal and Pritchett, 1993). A previous association study suggested a close relationship between *GABRB3*, the gene encoding the β3 subunit, and CAE (Feucht et al., 1999). As a result, the β3 subunit has been postulated to be associated with epilepsy, especially CAE. In addition, the chromosomal region 15q11-13, where *GABRB3* resides, is within the critical region of Angelman and Prader–Willi syndromes. The deletion of this region from the maternal chromosome is one cause of Angelman syndrome, which is often associated with absence-like seizures and autistic behavior. Conversely, mice lacking *GABRB3* present with epilepsy phenotypes and abnormal behavior similar to that seen in Angelman syndrome. These lines of evidence imply that *GABRB3* is involved in not only the etiology of epilepsy but also autism, a common comorbidity of epilepsy. In fact, mutations and variants of the β3 gene, *GABRB3*, have been associated with human autism in particular for maternal transmission (Cook et al., 1997; Delahanty et al., 2011). Furthermore, mutations of *GABRB3* and the gene encoding the β1 subunit, *GABRB1*, have been recently identified in the more severe forms of epilepsy, such as IS and Lennox–Gastaut syndrome (LGS) (Allen et al., 2013).

4.1 MUTATIONS OF *GABRB1*

4.1.1 Mutations in IS

A recent large-scale sequencing study examining a large cohort of epileptic encephalopathies revealed a heterozygous missense mutation of *GABRB1*, F246S, in a boy with IS (Allen et al., 2013). His seizure phenotype first presented with a focal seizure with conscious disturbance and evolved into IS associated with atypical absence, atonic, and myoclonic seizures. The patient also had severe psychomotor retardation. The pathomechanisms resulting from F246S have not been investigated, though an *in silico* analysis using Polyphen-2 category score indicated the mutation is probably damaging (Allen et al., 2013).

4.2 MUTATIONS AND VARIATIONS OF *GABRB3*

4.2.1 Mutations and Variations in CAE

A polymorphism at the promoter region of *GABRB3*, −897T>C, was associated with CAE (Urak et al., 2006). A previous association study identified a positive association between *GABRB3* and CAE (Feucht et al., 1999). A later study indicated that −897T>C is significantly associated with CAE and reduces the transcriptional ability of the promoter. This reduction seems to result from deficient binding of the neuron-specific transcriptional activator N-Oct-3 (Urak et al., 2006). Thus, a reduction of the β3 subunit can contribute to the appearance of CAE in the developing brain.

Three mutations of *GABRB3*, P11S, S15F, and G32R, were identified in four unrelated families in which the affected individuals showed the typical CAE phenotype in addition to other epileptic symptoms, such as eyelid myoclonus and grand mal seizures (Tanaka et al., 2008). Consistent with the typical CAE phenotype, all of these individuals experienced frequent absence seizures with the characteristic EEG findings (i.e., generalized 3-Hz spikes and waves during in childhood, but these clinical symptoms later remitted without neurological sequelae). The P11S mutation was identified in two unrelated families, whereas S15F and G32R were each identified in one family. These mutations were not found in 630 controls. P11S was also associated with 17 other Caucasian families with epilepsy and autism (Delahanty et al., 2011) and GGE in a French-Canadian family (Lachance-Touchette et al., 2010). However, an asymptomatic individual carrying P11S was identified, suggesting it is a rare SNP (Lachance-Touchette et al., 2010).

There are two translation starting sites in the *GABRB3* exons: exon 1a and exon 1 located downstream from 1a. Exons 1a and 1 are followed by the same exons 2–4, which result in the alternative transcriptional isoforms 2 and 1, respectively. In the fetal brain, isoform 2 is dominantly expressed compared to isoform 1. The P11S and S15 reside in exon 1a, whereas G32R resides in exon 2 and is located at the N-terminus of the β3 subunit (Kirkness and Fraser, 1993).

$GABA_A$ receptors reconstituted with a wild-type or mutant β3 subunit and α1 and γ2 subunits show different electrophysiological properties (Tanaka et al., 2008). Compared to the wild-type $GABA_A$ receptors, $GABA_A$ receptors harboring the *GABRB3* mutations showed attenuated Cl^- currents. This impairment in channel activity was attributed to hyperglycosylation resulting from the mutations (Tanaka et al., 2008).

An elaborate study that examined G32R challenges the idea that hyperglycosylation resulting from mutations reduces the surface expression of $GABA_A$ receptors harboring the mutation (Gurba et al., 2012). A study on reconstituted $GABA_A$ receptors in HEK293T cells that used expression of a mutant β3 subunit, β3(G32R), along with α1 or α3 and γ2 subunits, suggested that β3(G32R) increased the surface expression of β3 subunits and induced formation of binary αβ3 and homomeric β3 receptors, but reduced the number of ternary αβ3γ2L receptors. Consistent with the findings of the previous experiment, the β3(G32R) subunits were hyperglycosylated.

However, it is unlikely that hyperglycosylation of β3(G32R) subunits was responsible for changes in subunit surface expression. In addition, α1β3(G32R)γ2 showed a reduced macroscopic current density that could not be fully explained by changes in subunit expression levels or glycosylation. Single-channel recording revealed that α1β3(G32R)γ2 receptors had impaired gating with a decreased mean open time (Gurba et al., 2012). Such defects might have resulted from mutations that hindered subunit oligomerization and disrupted salt bridges at subunit interfaces.

4.2.2 Mutations in IS

A recent large-scale sequencing study on a large cohort of epileptic encephalopathies identified four heterozygous *de novo* missense mutations in IS and LGS: N25D, D35N, E109G, and Y302C. N25D was identified in a girl with IS who presented with only an IS seizure phenotype and EEG findings of hypsarrhythmia (Allen et al., 2013). Her prognosis was not reported. D35N, E109G, and Y302C were identified in individuals with LGS that had evolved from IS. The pathomechanisms underlying the mutations have not been characterized, though an *in silico* analysis using Polyphen-2 scoring indicated the mutations are probably damaging (Allen et al., 2013).

4.2.3 Animals with Aberrant β Subunits

Mice lacking *GABRB3* show epilepsy phenotypes and behavioral abnormalities corresponding to the characteristics of Angelman syndrome (Minassian et al., 1998), an imprinted disorder caused by maternal 15q11-13 or UBE3A deficiency (DeLorey and Olsen, 1999; DeLorey et al., 1998; Homanics et al., 1997). The critical region of Angelman syndrome, 15q11–13, encompasses *GABRA5* and *GABRG3*, which encode α5 and γ3 subunits, respectively, as well as *UBE3A* and *GABRB3*.

Both homozygous and heterozygous mice lacking *Gabrb3*, the mouse ortholog of *GABRB3*, exhibit EEG abnormalities that include bursts of abnormal slowing and irregular high-amplitude slow and sharp waves and small spikes in the background EEG. These EEG bursts coincided with behavioral abnormalities (immobility, fixed stare, and vibrissae twitching) appearing in the middle of activity and lasting seconds, or drowsiness with partial eye closure lasting several minutes. As the mice developed, they displayed high-amplitude spikes associated with clonic jerking of the head and forelimbs with an arching back (DeLorey and Olsen, 1999; DeLorey et al., 1998; Homanics et al., 1997). These findings verify an epilepsy phenotype for *GABRB3*-deficient mice. Furthermore, ethosuximide, an effective AED for human absence epilepsy, showed efficacy for the EEG abnormalities and clonic jerks, whereas carbamazepine, a contraindicated AED for generalized epilepsy and absence epilepsy, aggravated the EEG abnormalities (DeLorey and Olsen, 1999; DeLorey et al., 1998; Homanics et al., 1997). The mice also exhibited behavioral abnormalities such as learning and memory deficits, poor motor skills on a repetitive task, hyperactivity, and a disturbed rest–activity cycle, all of which correspond to the behavioral characteristics of Angelman syndrome (DeLorey and Olsen, 1999;