DeLorey et al., 1998; Homanics et al., 1997). Given that *GABRB3* is located in the same critical chromosomal region as Angelman syndrome, and epilepsy is often associated with Angelman syndrome, *GABRB3* is considered to play an important role in the pathomechanisms of epilepsy and the behavioral abnormalities in Angelman syndrome.

In accordance with the genetic mechanism of Angelman syndrome, a genomic imprinting abnormality of the maternal chromosomal, *Gabrb3*-deficient mice show parent-of-origin differences in β3 subunit expression, EEG activity, and behavior abnormalities. Thus, heterozygous mice lacking the maternal origin *Gabrb3* show a greatly reduced level of β3 subunits in the brain, which suggests the parent of origin can account for the pathology of underlying *Gabrb3* abnormalities. These findings imply that *Gabrb3* is also imprinted, and hence, human mutations of *GABRB3* in epilepsy could be influenced by the genomic imprinting in epileptogenesis.

5 MUTATIONS OF THE γ SUBUNIT

At present, more than a dozen mutations of *GABRG2*, the gene that encodes the γ2 subunit, have been associated with idiopathic epilepsy; most of these mutations are associated with FS. This indicates that the γ2 subunit plays an important role in the pathomechanisms of epilepsy. In fact, the γ2 subunit is considered one of the major receptor components that allow $GABA_A$ receptors to modulate phasic or synaptic transmission (Farrant and Nusser, 2005; Olsen and Sieghart, 2008; Sieghart and Sperk, 2002; Whiting, 2003). Moreover, it is believed that the γ2 and α subunits form a binding site for some AEDs, such as benzodiazepines, and allow the AEDs to potentiate or accelerate the depolarizing potential of $GABA_A$ receptors. The γ subunit is also crucial for the receptor's location in the synapse, as well as normal assembly and function of inhibitory synapses (Essrich et al., 1998; Fang et al., 2006).

Knock-out mice deficient in γ2 subunits have been generated. The homozygous knock-out mice died perinatally (Gunther et al., 1995), which underpins the crucial role this subunit plays. Heterozygous mice without benzodiazepine binding sites showed a decreased clustering of $GABA_A$ receptors in the hippocampus and cerebral cortex (Crestani et al., 1999). However, no epilepsy phenotype was observed in either the homozygous or heterozygous knock-out, whereas anxiety was documented in both heterozygous and knockdown mice for the γ2 subunit (Chandra et al., 2005; Crestani et al., 1999).

5.1 MUTATIONS IN CAE AND FS

At present, two mutations were identified in individuals with CAE and FS. One is a heterozygous missense mutation of *GABRG2*, R82Q, which is located within the benzodiazepine binding site. The R82Q mutation was identified in

a large family containing several individuals with idiopathic epilepsy, which were primarily FS and CAE phenotypes (Wallace et al., 2001). The other is a heterozygous splice site mutation, IVS6+2T>G, which is located at the splice donor site of intron 6. This mutation was identified in a brother and sister, whose phenotypes were absence epilepsy and FS, and in their father, whose phenotype was FS+.

The consequences of the R82Q mutation have been meticulously investigated by several groups. The mutation is located in the benzodiazepine binding site and causes low sensitivity to benzodiazepines (Bowser et al., 2002; Wallace et al., 2001). Thus, it was initially deduced that receptors harboring this mutation lose their inhibitory functions through endozepine, a putative benzodiazepine-like compound, thereby resulting in the epilepsy phenotype (Wallace et al., 2001). However, this pathomechanism has been debated (Bianchi et al., 2002; Macdonald et al., 2003), and several studies identified retention of the mutant receptors in the ER. This finding may be attributed to improper oligomerization caused by the mutant γ subunit, as the R82Q mutation is believed to be located at the interface with β2 subunits (Hales et al., 2005). Hence, the main electrophysiological deficit of this mutant $GABA_A$ receptor is abnormal intracellular trafficking of channel molecules followed by degradation of the molecules (Kang and Macdonald, 2004; Macdonald et al., 2004; Sancar and Czajkowski, 2004). A dominant-negative effect was suggested as the pathomechanism supporting the dominant inheritance of the family's epilepsy phenotypes (Kang and Macdonald, 2004; Macdonald et al., 2004; Sancar and Czajkowski, 2004).

A reduction in the number of mutant $GABA_A$ receptors on the cell membranes of cultured hippocampal neurons might have been precipitated by a temperature increase. Such an effect is presumably due to temperature-sensitive trafficking and/or altered endocytosis of the mutant $GABA_A$ receptors harboring the R82Q mutation (Kang et al., 2006). This finding may indicate a pathomechanism underlying FS in individuals with this mutation. However, this theory was not supported by the findings of an *in vivo* experiment with knock-in mice bearing the R82Q mutation (Hill et al., 2011; Tan et al., 2007). Endocytosis of the mutant $GABA_A$ receptors was accelerated, which may have contributed to the reduced number of $GABA_A$ receptors available at the synaptic membrane. Intriguingly, accelerated endocytosis of the mutant receptors could be attenuated by the receptor's agonists, which implies a therapeutic measure for epilepsies resulting from *GABRG2* mutations (Chaumont et al., 2013).

Another study proposed a new pathological effect of the R82Q mutation that alters the subunit composition of $GABA_A$ receptors expressed on the cell membranes of cultivated hippocampal neurons, in addition to the ER retention of the mutated $GABA_A$ receptors (Frugier et al., 2007). However, the findings of that study do not support the dominant effect of the mutation in the alteration (Frugier et al., 2007). This alteration in the subunit composition may be the basis for the different consequences of the mutation in the phasic and tonic inhibitions mediated by the mutant $GABA_A$ receptor, which was shown in another study with cultured hippocampal

neurons (Eugene et al., 2007). Thus, the R82Q mutation does not exert a dominant-negative effect on either synaptic or phasic inhibition, but reduces perisynaptic or tonic inhibition. This finding of reduced tonic inhibition challenges previous findings that tonic inhibition is increased in thalamocortical neurons in an animal model of absence epilepsy (Cope et al., 2009). However, given that some $GABA_A$ receptors with the R82Q mutation have reduced or altered subunit compositions, compensatory mechanisms may increase the number of $GABA_A$ receptors containing certain subunits, such as the $\alpha\beta\delta$ subtype that mediates tonic inhibition. The $GABA_A$ receptors produced from such compensation might generate an overall excess of tonic inhibition. Alternatively, any impairment in tonic inhibition mediated by $GABA_A$ receptors may result in absence epilepsy, regardless of increases or decreases in tonic inhibition.

Knock-in mice bearing the R82Q mutation have been used to further examine the pathomechanisms caused by this mutation. These knock-in mice exhibit an epilepsy phenotype similar to that in human CAE and a thermal sensitivity for seizures (Tan et al., 2007), as shown in the pedigrees including individuals with the same mutation (Marini et al., 2003). EEG recordings of the knock-in mice showed 6- to 7-Hz spike and wave discharges on their behavioral arrest, which are reminiscent of the 3-Hz spike and wave discharges that occur during absence seizures in CAE. Furthermore, the 6- to 7-Hz spike and wave discharges respond well to ethosuximide, one of the primary drug treatments for CAE (Tan et al., 2007). Therefore, these knock-in mice are considered to represent the *in vivo* phenomena present in the CNS of individuals with the mutation.

Intracellular retention and a reduced surface expression of $\gamma 2$ subunits bearing the R82Q mutation were also identified in the knock-in mice, although the trafficking of the $\alpha 1$ subunit was not affected by the mutant $\gamma 2$ subunit (Tan et al., 2007). The discrepancy between the findings of *in vitro* studies with cultivated cells and *in vivo* studies with the knock-in mice indicates that trafficking of the $GABA_A$ receptor is influenced by complicated mechanisms. A small reduction in mIPSCs was identified in layer II/III cortical neurons, but not in thalamic neurons. This suggests that a subtle reduction in the number of cortical neurons maybe an underlying mechanism of absence epilepsy. This hypothesis was supported by findings obtained from transcranial magnetic stimulation analyses on patients harboring the mutations (Fedi et al., 2008). Individuals with the R82Q mutation underwent an examination of benzodiazepine sensitivity using ^{11}C-flumazenil positron emission tomography, which revealed that these individuals have poor benzodiazepine binding (Fedi et al., 2006). This finding seems to conflict with the accepted R82Q pathology, which includes oligomerization followed by receptor trafficking abnormalities, but not deficient benzodiazepine binding.

Knock-in mice bearing the R82Q mutation also display thermally sensitive seizures and, therefore, they may be a good model for investigating the molecular pathomechanisms of FS (Tan et al., 2007). Using the R82Q mutation for this purpose is of particular interest because the penetrance of CAE and FS phenotypes resulting from the R82Q mutation appears different in large pedigrees where the mutation was

first reported (Marini et al., 2003; Wallace et al., 2001). In fact, experiments with knock-outs of *Gabrg2* and knock-ins with the R82Q mutation that were generated with different mouse strains indicate that even a single mutation can result in distinct seizure phenotypes, which has been anticipated from the point of view of clinical genetics with human disorders (Reid et al., 2013).

Intriguingly, incremental temperature did not reduce mIPSCs in layer II/III of brain slice preparations obtained from knock-in mice bearing the R82Q mutation, but increased the inhibitory currents (Tan et al., 2007). Similarly, another study that analyzed GABAergic mIPSCs in brain slice preparations from knock-in mice found that elevated temperature increased mIPSC amplitudes. Moreover, there were no temperature-dependent differences in the density of receptors containing the γ2 subunit. This finding challenges the findings from an earlier study in which temperature increases precipitated the retention of the mutant $GABA_A$ receptors, which was proposed as the mechanism underlying FS associated with the γ2 subunit mutations (Kang et al., 2006). This is an example of discrepancies between the findings from *in vitro* and *in vivo* experiments that may be reconciled with further experiments.

Knock-in mice bearing the R82Q mutation provide information that could be an indicator for seizure susceptibility. The MRI findings of knock-in mice showed increases in the volume and neuronal density of the granule cell layer of the dentate gyrus before seizure activity. Similar morphological changes, if they are detectable, may predict seizure susceptibility in individuals with the R82Q mutation or other genetic predisposition for epilepsy (Richards et al., 2013).

Knock-in mice bearing the R82Q mutation also provide insight into how the mutation may affect seizure development. Tetracycline-controlled conditional expression of the R82Q mutation showed that seizure development could be significantly suppressed when the mutation expression is suppressed during development. This suggests that there is a period in which the sensitivity to epileptogenesis caused by the genetic abnormality is increased in developing epilepsy (Chiu et al., 2008).

The developmental influence of the R82Q mutation has been explained recently in the context of $GABA_A$ receptor development. This description states that immature $GABA_A$ receptors can generate a giant depolarizing potential (GDP), which is important for the developing brain. GDPs can be generated in the developing brain where NKCC1, a transporter that increases intracellular Cl^- concentration, dominates the counteractions of KCC2 (Fukuda, 2005). The R82Q knock-in mice, however, showed a decrease in GDPs. Bumetanide, an inhibitor of NKCC1, could reduce GDP mimicking and attenuate GDPs in knock-in mice and, likewise, induce seizures in the bumetanide-treated wild-type neonatal pups (Vargas et al., 2013). This finding warns against using bumetanide in neonates, despite that bumetanide is one of the candidate drugs for controlling seizures in neonates (Dzhala et al., 2005).

Another mutation, IVS6+2T>G, was identified in brothers with CAE and FS. This mutation is a heterozygous transversion of a nucleotide at a splice donor site of intron 6 and is believed to encode a nonfunctional γ2 subunit (Kananura et al., 2002). The splicing resulting from the mutation was examined using artificial bacterial chromosomes bearing the mutation with transfected cultivated cells and

transgenic mice (Tian and Macdonald, 2012). This study revealed the mutation results in a mutant γ2 subunit with aberrant amino acids followed by a premature termination codon. Although the mutant transcripts undergo nonsense-mediated decay, the escaped mutant γ2 subunits are stable. The mutant γ2 subunits are not expressed on the cell surface and are retained in the ER, where they are oligomerized with α1 and β2 subunits. As a result, the mutation reduces GABAergic inhibition and may also induce ER stress.

5.2 MUTATIONS IN GEFS+

Several mutations of GABRG2 have been identified in individuals with simple FS and GEFS+, a clinical subset of FS also known as autosomal dominant epilepsy febrile seizure plus (ADEFS+) (Ito et al., 2002). Individuals with GEFS+ suffer from FS under the age of 6 years and thereafter develop various types of epilepsies and non-FSs. At present, one missense mutation, K328M (Baulac et al., 2001), and three nonsense mutations, R136* (Lachance-Touchette et al., 2011), W429* (Sun et al., 2008), and Y444Mfs51* (Tian et al., 2013), were associated with GEFS+.

K328M is a heterozygous mutation identified in a pedigree of GEFS+. Electrophysiological study of reconstituted $GABA_A$ receptors in the *Xenopus* oocyte demonstrated that the mutation reduced GABA-induced currents (Baulac et al., 2001). Single-channel recording revealed fast deactivation of the $GABA_A$ receptor bearing the mutant γ2 subunits, whereas intracellular trafficking of the mutant receptor was unaffected in HEK cells when compared to receptors bearing the γ2(R82Q) mutation (Bianchi et al., 2002; Macdonald et al., 2003). These changes may be attributed to the low channel-opening equilibrium constant of the mutated receptor (Ramakrishnan and Hess, 2004). These findings have been confirmed by those from other studies in that the K328M mutation affects the channel gating properties of the $GABA_A$ receptor, but does not perturb the receptor trafficking (Bouthour et al., 2012; Hales et al., 2006).

R136*, a heterozygote nonsense mutation, was recently identified in a family in which most individuals with the mutation showed FS. Various epilepsy phenotypes were present in the family, which is consistent with the diagnosis of GEFS+, even though there were phenocopies in the family (i.e., individuals with epilepsy but without the mutation) (Johnston et al., 2014). Interestingly, two individuals with the mutation were diagnosed with autistic spectrum disorder (Johnston et al., 2014). This may indicate that the $GABA_A$ receptor is an important player in both epilepsy and autism. Like other γ2 mutants, γ2 subunits bearing the R136* mutation result in intracellular trafficking defects that include ER retention of the mutant receptors and reduced expression of the $GABA_A$ receptors containing the γ2 subunit, whereby GABAergic inhibition is impaired (Johnston et al., 2014; Kang et al., 2013).

A recent sequencing study on a cohort of 500 patients with epileptic encephalopathy identified a boy with a heterozygous missense mutation, R323Q, whose phenotype corresponded to GEFS+ with FS at the age of 8 months, followed by absence seizures, atonic seizures, myoclonic jerks, and tonic–clonic seizures. The

mutation was *de novo*, which has not been examined to determine the pathological consequences of the mutation (Carvill et al., 2013).

W429*, a heterozygote nonsense mutation, was identified in a family in which the GEFS+ phenotypes was inherited with high penetrance (Sun et al., 2008). This mutation also caused intracellular trafficking abnormalities and reduced cell surface expression of $GABA_A$ receptors. This results in ER retention of the mutant γ2 subunit followed by ERAD and possibly ER stress (Kang et al., 2013).

Y444Mfs51* is a heterozygous one-base deletion mutation that extends the C-terminus with 51 aberrant amino acids followed by a stop codon (Tian et al., 2013). This mutation was well segregated in an Italian family among individuals with either the GEFS+ phenotype or frequent FS episodes. The mutation results in a significant reduction of GABA-evoked currents, which is caused by retention of the mutant γ2 subunit. The mutant γ2 subunit is translated as a stable protein but is not transferred to the cell membrane. Consequently, α and β subunits form receptors without the mutant γ2 subunit.

5.3 MUTATIONS IN DRAVET SYNDROME

Dravet syndrome is one of the most devastating forms of epilepsy. The majority of its genetic cases include mutations of *SCN1A*, the gene encoding the α1 subunits of the neuronal sodium channel, $Na_v1.1$. Because GEFS+ is caused by missense mutations of *SCN1A*, and Dravet syndrome is frequently associated with FS and temperature-induced seizures, Dravet syndrome and GEFS+ are considered to belong to the same disease spectrum that includes FS as the mildest phenotype (Hirose et al., 2002a,b, 2003, 2005).

Other known causes of Dravet syndrome include heterozygous mutations of *SCN2A*, the gene encoding the α2 subunit of the sodium channel, $Na_v1.2$ (Shi et al., 2009) and homozygous mutations of *SCN1B*, the gene encoding the β1 subunit of sodium channels (Ogiwara et al., 2012; Patino et al., 2009). Nevertheless, such mutations, including those of *GABRG2*, are considered minor causes of Dravet syndrome (Hirose et al., 2002a,b, 2003, 2005, 2013; Shi et al., 2012).

At present, Dravet syndrome is thought to result from two heterozygous nonsense mutations, Q40* and Q390* (Harkin et al., 2002; Ishii et al., 2014). A heterozygous nonsense mutation of *GABRG2*, Q40*, was identified in dizygotic twin girls whose phenotype was compatible with that of Dravet syndrome. The elder sister died at the age of 3 years and 5 months, which allowed for postmortem examination of brain specimens (Ishii et al., 2014). Interestingly, this mutation was also detected in the asymptomatic father, but not in their mother who had several seizure episodes in early childhood. This implies genetic modifiers for Dravet syndrome besides the Q40* mutation (Ishii et al., 2014).

Several studies revealed that the Q40* mutation indeed causes a premature stop at the first amino acid after the signal peptide and interferes with $GABA_A$ receptor trafficking and receptor retention in the ER. This is quite surprising because such stop codons located on the 5′ end of mRNA are commonly believed to induce

NMD; thus, one may postulate that the mutant γ2 subunit would be eliminated by the NMD. Although the mutant γ2 subunit induced NMD, mRNA escaped from NMD is likely to be translated to exert a dominant-negative effect on $GABA_A$ receptor sorting to the membrane (Huang et al., 2012; Ishii et al., 2014; Kang et al., 2013). The accumulation of mutant receptors could be a burden on the ER (i.e., ER stress followed by apoptosis) (Hirose, 2006; Kang and Macdonald, 2009).

In accordance with these *in vitro* experiments, immunohistochemical examinations of the brain specimens obtained from one of the patients with anti-α1 and -γ2 subunits demonstrated fine granules in neuron cell bodies and neutrophils, thus indicating aggregations of $GABA_A$ receptors in the neurons of patients with otherwise normal brain structures (Ishii et al., 2014).

Similarly, Q390* was identified in a male with Dravet syndrome in a large Austrian family containing a number of affected individuals that showed simple FS and myoclonic astatic epilepsy. The same mutation was detected in the mother and an elder brother who experienced simple FS, but they did not present with the Dravet syndrome phenotype (Harkin et al., 2002).

Reconstituted $GABA_A$ receptors in *Xenopus* oocytes consisting of wild-type α1, β2, and mutant γ2 subunits failed to show GABA-evoked currents. Retention of the mutant receptor in the ER was detected in HEK cells transfected with α1, β2, and mutant γ2 subunits (Harkin et al., 2002). This trafficking deficiency is temperature dependent *in vitro*, which may indicate the mechanisms underlying fever-sensitive phenotypes in individuals with Q390* (Kang et al., 2006). The mRNA of the mutant Q390* γ2 is indeed translated from mRNA escaping from NMD (Kang et al., 2006, 2009a, 2013). The $GABA_A$ receptors oligomerized with mutant Q390* γ2 that are trapped in the ER undergo ERAD through the ubiquitin-proteasome system (Kang et al., 2009a). An *in vitro* study with cultured neurons showed that the mutant $GABA_A$ receptors form aggregates that are resistant to the degradation process. This finding is reminiscent of the fine granules found in the brain specimens of patients with the Q40* mutation, another Dravet syndrome associated nonsense mutation (Ishii et al., 2014). Thus, protein aggregation often seen in neurodegenerative diseases could be one of the pathogeneses of the more severe forms of epilepsy (Hirose, 2006).

Indeed, ER retention of the mutant γ2 subunits trapping the α1 subunit was verified not only with cultured cells but also with knock-in mice (Kang et al., 2013). In addition, other deficiencies resulting from the Q390* mutation, such as trafficking defects, over ubiquitination, and ER stress, were also confirmed and considered a modifier for the severity of epilepsy caused by γ2 subunit mutations. Thus, it is anticipated that a larger number of mutant γ2 subunits retained in the ER result in a more severe phenotype of epilepsy, such as Dravet syndrome (Kang et al., 2013).

5.4 MUTATIONS IN IDIOPATHIC GENETIC GENERALIZED EPILEPSY

Two heterozygous missense mutations of *GABRG2*, N79S and P83S, were identified in other forms of GGEs or idiopathic generalized epilepsy (Berg et al., 2010), which may not be classified as epilepsy syndromes (Lachance-Touchette et al., 2011;

Shi et al., 2010). N79S was identified in a genetic analysis of a large cohort of 140 pediatric patients with various forms of epilepsy. The patient harboring the N79S mutation was a girl who had several episodes of GTCSs. She exhibited the typical seizure and EEG phenotype and a susceptibility to AED for GTCS. A functional study on the electrophysiological properties of $GABA_A$ receptors reconstituted in HEK cells showed a change in the concentration–response relationship for GABA that makes the mutant receptor less responsive to GABA at lower concentrations compared to the wild-type $GABA_A$ receptor (Migita et al., 2013).

P83S was identified in individuals with GGE across three generations in a French-Canadian family. However, no differences were identified in the mutant GABA receptors with regard to their surface expression or functional properties (Lachance-Touchette et al., 2011). Thus, further study on the relationship between this mutation and the epilepsy phenotype is necessary.

6 MUTATIONS OF THE δ SUBUNIT

The δ subunit is an important constituent of the $GABA_A$ receptors mediating tonic inhibition in neurons. This is in contrast to the γ subunits that are a major component of the receptors mediating phasic inhibition (Bai et al., 2001; Saxena and Macdonald, 1994; Stell et al., 2003; Wei et al., 2003). At present, three genetic variations (E177A, R220C, and R220H) of *GABRD*, the gene encoding δ subunit, have been associated with epilepsy in a cohort study with 72 unrelated GGE, 65 unrelated GEFS+, and 66 unrelated FS patients (Dibbens et al., 2004).

E177A is a heterozygous missense mutation identified in a GEFS+ family in which the affected individuals presented with FS, FS+, and an unclassified epilepsy. Even so, the mutation did not segregate monogenetically and therefore might be an allele for the seizure susceptibility underlying polygenic epilepsy. Electrophysiological experiments with reconstituted $GABA_A$ receptors in HEK cells transfected with cDNA of wild-type α1, β2, and δ subunits demonstrated reduced GABA-evoked currents in mutant receptors containing α1, β2, and mutant δ subunits (Dibbens et al., 2004). The effect of the mutation on the receptor cell surface expression and single-channel gating properties were also studied (Feng et al., 2006). Surface expression of $GABA_A$ receptors containing α4, β2, and mutant δ subunits was not altered when it was examined with wild-type $GABA_A$ receptors mimicking the heterozygous mutation in these patients. The mean open duration of mutant receptors containing the mutant δ subunit was decreased compared to wild-type receptors (Feng et al., 2006).

The R220C mutation was identified in a small family with GEFS+. The mutation did not alter GABA EC_{50} or the maximal currents of $GABA_A$ receptors containing α1, β2, and mutant δ subunits (Dibbens et al., 2004). Thus, the functional significance of the R220C mutation in the pathomechanisms of GEFS+ remains unclear.

The R220H variation was identified in a small family in which the affected individuals presented with JME. Because this was also found in the general

population, R220H is considered a seizure susceptible allele that contributed to the pathogenesis of JME in this family. However, this hypothesis was not supported by an independent association study of genetic variation performed with 562 unrelated German patients with GGE and 664 healthy population controls (Lenzen et al., 2005). An electrophysiological study of $GABA_A$ receptors constituted with the $\gamma 2$ subunit bearing R220H revealed a reduced GABA-induced maximal current in $GABA_A$ receptors, whereas R220H did not change the GABA EC_{50} for the receptors. However, surface expression of the mutant $GABA_A$ receptors was affected. Single-channel analyses showed a reduction in the channel-opening duration. Consequently, insufficiencies of the mutant receptors reduce hyperpolarization potential and, therefore, GABAergic transmission.

7 THERAPEUTIC IMPLICATIONS OF $GABA_A$ RECEPTOR MUTATIONS

Mutations of $GABA_A$ receptor subunits have been associated with GGE and FS. Furthermore, there is evidence that deficient GABAergic neurons play a crucial role in the development of epilepsy. Thus, GABA receptors are likely key players in the pathomechanisms of epilepsy. Keeping this in mind, $GABA_A$ receptors should be considered for their potential in therapeutic intervention.

Besides the mutant $GABA_A$ receptors described above, one line of evidence indicates that $GABA_A$ receptors and GABAergic neurons both directly and indirectly affect the development of epilepsy and FS. Recent studies that used genetically engineered animals or induced pluripotent stem cell (iPS cell)-derived neurons have demonstrated a close relationship between GABAergic neurons and epileptogenesis (Higurashi et al., 2013).

Dravet syndrome is caused mainly by mutations of *SCN1A*, the gene encoding the $\alpha 1$ subunit of $Na_v1.1$ sodium channels, yet a small portion of cases of this syndrome can cause $GABA_A$ receptor mutations. Studies with mice bearing *SCN1A* mutations have demonstrated that the dysfunctions of the mutated channels have been observed predominantly in GABAergic neurons (Chen et al., 2004; Ogiwara et al., 2007, 2013). The selective disruption of channel functions in inhibitory GABAergic and excitatory glutamatergic neurons have provided compelling evidence that the relatively selective dysfunctions of GABAergic inhibitory neurons are the main cause of Dravet syndrome (Ogiwara et al., 2013). Recent experiments on neurons derived from patient iPS cells have confirmed the pathomechanism in human neurons (Higurashi et al., 2013).

Mutations of neural acetylcholine receptors cause autosomal dominant frontal epilepsy characterized by seizures occurring exclusively during sleep (Hirose et al., 1999, 2000, 2002a,b, 2003, 2005; Kurahashi and Hirose, 1993). A study on transgenic rats harboring one of these mutations revealed the impaired GABAergic inhibitory transmission was most evident during sleep and preceded the seizures during sleep (Zhu et al., 2008). A recent study showed that

furosemide, a loop diuretic, has a prophylactic effect on seizure development in a transgenic rat model of epilepsy (Yamada et al., 2013). Furosemide inhibits NKCC1, which subsequently decreases intracellular Cl^- concentration and accelerates GABAergic inhibitory activity. Thus, administration of furosemide prior to seizure development prevented seizure activity to some extent in the rat model. Once the seizure activities were prevented, furosemide was discontinued. This is consistent with the finding that there is a period for epileptogenesis in knock-in mice with the R82Q mutation of the γ2 subunit (Chiu et al., 2008). However, bumetanide, another loop diuretic, may precipitate seizures in the knock-in mice as the GDPs are decreased (Vargas et al., 2013). Nevertheless, modulation of $GABA_A$ receptors or GABAergic neurons is expected to be a therapeutic measure for epilepsy in the developing brain.

A stop codon for *GABRG2* has been shown to be repaired *in vitro* using the "read-through" phenomenon induced by aminoglycosides, such as gentamicin. Thus, some forms of epilepsy resulting from mutations may be curable (Huang et al., 2012). Attempts to repair stop codons have been implemented in other disorders. Such attempts include inducing "read-through" (Welch et al., 2007) and "exon skipping" (Malueka et al., 2011; Matsuo et al., 1991; Nishida et al., 2011). The rescue of premature stop codons by certain drugs might be a new treatment for epilepsy resulting from mutations that create premature stop codons.

One of the lessons learned from research on $GABA_A$ receptors mutations is that legitimate, genetically engineered animal models are a prerequisite for developing effective AEDs based on the actual pathomechanisms of epilepsy. First, there are some discrepancies between the findings obtained from *in vitro* experiments performed with cultivated cells and *in vivo* experiments performed with genetically engineered animal models. For example, regarding the pathomechanisms of FS, an *in vitro* study showed that high temperature precipitated ER retention of the mutant γ2 subunit, but this finding has not been supported *in vivo* with the corresponding knock-in mice. Second, conventional epilepsy models, which are mainly acute seizure models, do not represent the actual pathomechanisms of epilepsy in the CNS. For example, the brain uptake of diazepam and phenytoin was considerably different between acute seizure models generated with pentylenetetrazole and wild-type mice, whereas there was no such difference with the genetic absence epilepsy model bearing the R82Q mutation of the γ2 subunit (Nicolazzo et al., 2010).

8 CONCLUSIONS

The $GABA_A$ receptors are one type of ion channels with genetic mutations or variations that underlie epilepsy. Contrary to the mutations of other ion channels that affect channel functions, mutations of $GABA_A$ receptor subunits show more complex pathomechanisms of epilepsy. Thus, the pathomechanisms resulting from such mutations are closely associated with cell mechanisms such as ERAD, NMD,