CA), anti-PDI antibody (Cat# SPA-890, StressGen Biotechnology, Victoria, BC, Canada), and anti-GFP antibody (Cat# AB16901, Millipore).

Statistical analysis

Data were expressed as mean ± SD. Differences in the distribution of $GABA_A$ receptors among genotypes were analyzed by analysis of variance with Scheffe's multiple comparison using GLM (general linear model). Statistical significance of electrophysiological data was determined by one-way ANOVA, followed by Dunnett's method as post hoc test. All statistical analyses were performed using the Statistical Analysis System software (version 9.2, SAS Institute Inc., Cary, NC). A P value of <0.05 denoted the presence of significant statistical difference.

Results

Pedigree of twins with Dravet syndrome and a *GABRG2* mutation (p.Q40X)

Genetic analyses were performed for several Dravet syndrome candidate genes. The selected genes are known to encode components of neuronal sodium channels (*SCN1A, 2A, 1B, 2B*) or $GABA_A$ receptors (*GABRA1, B2, G2*) and contain mutations associated with both Dravet syndrome and GEFS+. A single *GABRG2* mutation (c.118C>T, based on a human *GABRG2* cDNA: RefSeq NM_198904) was identified in dizygotic twin girls with Dravet syndrome and in their apparently healthy father, but not in their mother who experienced seizures during childhood (Fig. 1A). No other mutation was detected within the examined regions of *GABRG2* or the other genes. The mutation was heterozygous and not found in other family members, including the mother with history of epilepsy and 182 healthy Japanese volunteers (Fig. 1B). The mutation was deduced to be associated with premature termination codons (PTCs) at position 40 of the GABRG2 molecule (p.Q40X) (Fig. 1D). The resultant mutated $\gamma2$ subunit was truncated at the N terminus after a very short amino acid sequence (Fig. 1C).

Electrophysiological properties of $GABA_A$ receptor bearing p.Q40X in $\gamma2$ subunit

To gain insight into the functional consequences of the p.Q40X mutation in the $\gamma2$ subunit, we analyzed GABA-mediated currents in HEK293 cells expressing $\alpha1\beta2$, wild type (WT) $\alpha1\beta2\gamma2$, heterozygous $\alpha1\beta2\gamma2\gamma2$ (Q40X), or homozygous $\alpha1\beta2\gamma2$ (Q40X) $GABA_A$ receptors (Fig. 2). HEK293 cells expressing WT subunits produced a robust inward current in response to GABA. $GABA_A$ receptors consisting of $\alpha1$ and $\beta2$ subunits only also produced an inward current in response to GABA. However, peak currents and current density were decreased compared to those of $GABA_A$ receptors consisting of WT $\alpha1\beta2\gamma2$. The inward currents and current density of homozygous $\alpha1\beta2\gamma2$ (Q40X) were intermediate between WT $\alpha1\beta2\gamma2$ and heterozygous $\alpha1\beta2\gamma2\gamma2$ (Q40X) $GABA_A$ receptors. This result suggested that mutant $\gamma2$ (Q40X) had haploinsufficiency effect. Interestingly, peak currents and current density obtained from $GABA_A$ receptors were decreased when cDNAs of the mutant $\gamma2$ (Q40X) were transfected with those of other components of $GABA_A$ receptor, $\alpha1$ and $\beta2$ subunits. This result suggests that the effect of the mutant $\gamma2$ (Q40X) subunit on the $GABA_A$ receptor function was loss of function or dominant-negative suppression when current densities were compared.

Immunohistochemistry of patient brain

Immunohistochemical assay of the patient and control brain specimens using anti-$\alpha1$ subunit and anti-$\gamma2$ subunit antibody was performed to determine the change in $GABA_A$ receptor subunit (Fig. 3). In the patient brain specimens immunostained for $\gamma2$ subunits, granules were observed in neuronal soma and neuropils. The same granules were also observed in neuronal soma and neuropils stained with antibody against $\alpha1$ subunits of $GABA_A$ receptor. These findings suggest that $GABA_A$ receptor harboring the mutant $\gamma2$ (Q40X) subunit form aggregates in brain cells. It has been reported that deficient $GABA_A$ receptors precipitate in the ER (Harkin et al., 2002; Kang and Macdonald, 2004).

Localization of $\alpha1$ subunits in HEK293T

To map the intracellular localization of the mutant molecules, HEK293T cells, were transiently microinjected with pcDNA-3.1 vectors bearing either WT $\alpha1^{myc}\beta2\gamma2$ or homozygous $\alpha1^{myc}\beta2\gamma2$ (Q40X) cDNA. These cells were subsequently immunostained for *myc* to visualize the channel proteins. In HEK293T cells, the distribution of the mutant subunits [homozygous $\alpha1^{myc}\beta2\gamma2$ (Q40X)] associated with Dravet syndrome differed from that of WT protein. The $\alpha1$ subunit was expressed on the cell surface and in the ER, as shown by co-staining for the ER marker protein disulfide isomerase (PDI) at 6 h after microinjection (data not shown). The combinations of receptors produced from $\alpha1$ and $\beta2$ subunits are capable of exiting the ER and accessing the cell surface. Therefore, the $GABA_A$ receptors expressed on the cell surface are likely to be those subunits that could elude the trafficking disruption due to the mutant $\gamma2$ (Q40X) molecules.

We next considered the role of $\gamma2$ subunits in the cells. These subunits are known to be important in conferring benzodiazepine sensitivity on $GABA_A$ receptors and in controlling the synaptic targeting of these receptors (Essrich et al., 1998; Gunther et al., 1995). Furthermore, the $\gamma2$ subunits participate in the translocation of $GABA_A$ receptor to the cell membrane (Keller et al., 2004; Kittler et al., 2002; Moss and Smart, 2001; Sarto et al., 2002), and to act with $GABA_A$ receptor-associated protein (GABARAP) in other intercellular transportation of the GABA receptor (Kanematsu et al., 2002; Kittler et al., 2001, 2002; Kneussel, 2002; Wang et al., 1999). The above studies suggested that the *GABRG2* early truncation mutation (p.Q40X) could induce a delay in $GABA_A$ receptor trafficking to the cell surface.

To investigate the effect of the mutated $\gamma2$, we studied the time course of trafficking of $GABA_A$ receptor harboring the mutated $\gamma2$ after microinjection of the relevant cDNAs and the effect of dose of the mutated $\gamma2$

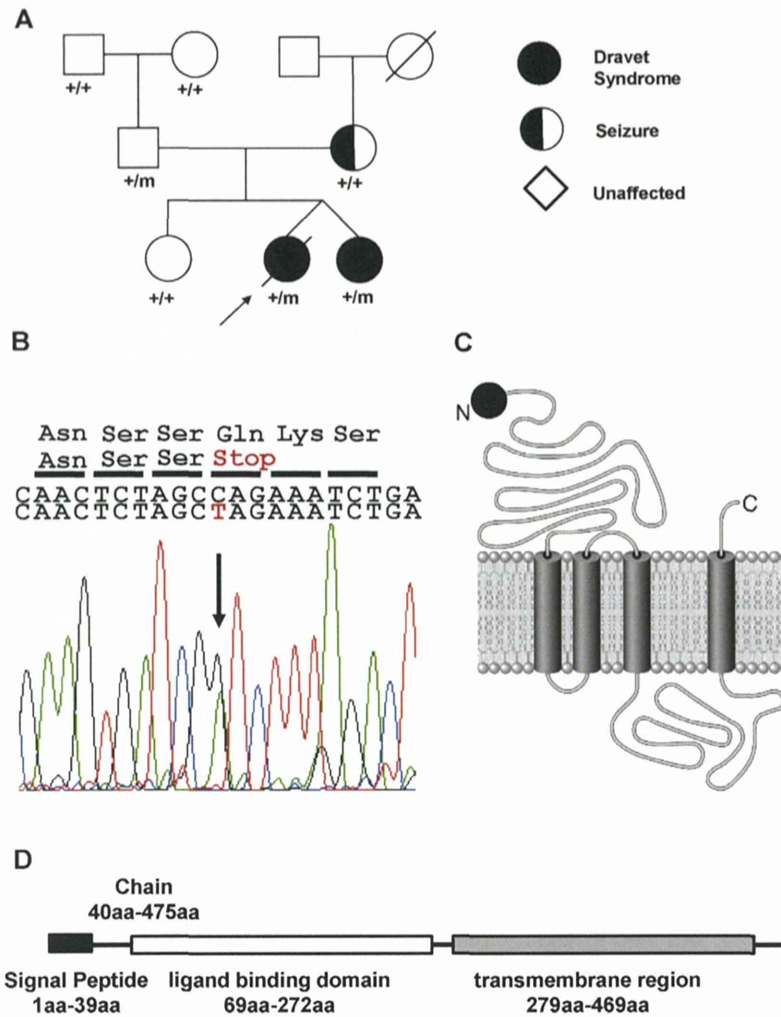

Figure 1 (A) Pedigree of the patient with Dravet syndrome and mutation analysis. *Arrow*: the proband, *squares*: males, *circles*: females. +: wild type allele, m: allele found to have a p.Q40X mutation. The mutation c.118C>T: p.Q40X in *GABRG2*, which was detected in dizygotic twin girls with Dravet syndrome and their apparently healthy father, but not in their mother who experienced seizures in childhood. The father's mutation was *de novo*. (B) Nucleotide sequence of the relevant region of *GABRG2* in the patient. Genomic DNA from the patient was amplified for exon 2 of *GABRG2* by PCR. The nucleotide sequences of the products were determined sequencing, in the 5′–3′ direction. *Arrow*: nucleotide 118, where three nucleotides were changed by the mutation. (C) Schematic representation of the GABRG2 protein product showing the position of the p.Q40X mutation. (D) Mutational position in the GABRG2 protein (arrow). *Solid square*: Signal peptide located from 1 to 39 amino acid, *open square*: Ligand binding domain, *gray square*: Transmembrane domain. The mutation p.Q40X is located directly below the signal peptide, resulting in a truncated GABRG2 protein with complete loss of the ligand binding domain and the transmembrane domain.

(i.e., heterozygous or homozygous of the mutated $\gamma 2$). We carried out a short (4 h) and long (18 h) incubations of WT $\alpha 1^{myc}\beta 2\gamma 2$-, heterozygous $\alpha 1^{myc}\beta 2\gamma 2\gamma 2$ (Q40X)-, and homozygous $\alpha 1^{myc}\beta 2\gamma 2$ (Q40X)-injected cells. Microscopic analysis of these cells demonstrated the majority of the $\alpha 1^{myc}$ subunit for WT, heterozygous, and homozygous receptors colocalized with PDI in the case of shorter incubation (4 h) after microinjection (Fig. 4A), and only WT cells had identifiable $\alpha 1^{myc}$ subunit colocalized with the membrane. In contrast, the majority of the $\alpha 1^{myc}$ subunit for heterozygous and homozygous receptors was retained in the ER even in the case of longer incubations (18 h) after microinjection (Fig. 4B). Quantification of the localization of the $\alpha 1^{myc}$ subunit was done by the manual counting of cells showing myc staining colocalized with the membrane or ER, and each quantification procedure was replicated three times. For the WT receptor, 66 cells (15, 21, and 30 cells in each of the three independent replications) were counted in the short incubation condition (i.e., 4 h), and 81 (40, 23, and 18) cells in the long incubation condition (i.e., 18 h); all these cells expressed the $\alpha 1^{myc}$ subunit. In the short incubation condition, the $\alpha 1^{myc}$ subunits were found predominantly on the membrane in 15 of the cells (4, 7, and 4 in each of the three independent replications), whereas the $\alpha 1^{myc}$ subunits were found predominantly in the ER of the 51 remaining cells (11, 14, and 26). In contrast, in the long incubation condition, the $\alpha 1^{myc}$ subunits were found predominantly on the membrane in 61 of the cells (22, 21, and 18 in each of the three independent replications), whereas the $\alpha 1^{myc}$ subunits were

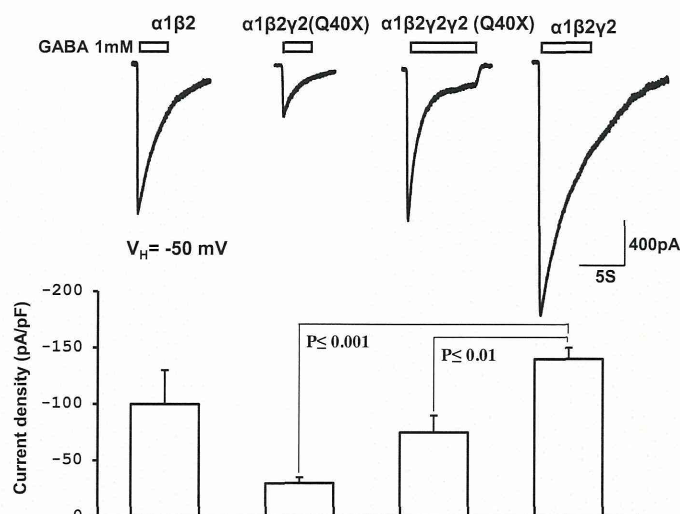

Figure 2 Electrophysiological properties of $GABA_A$ receptor $\gamma 2$ subunit bearing p.Q40X mutation. Electrophysiological studies were performed to evaluate the function of $GABA_A$ receptors reconstituted with $\alpha 1$, $\beta 2$, and $\gamma 2$ subunits in HEK cells. Additional transfection of mutated $\gamma 2$ (Q40X) suppressed GABA-induced current amplitudes for the $GABA_A$ receptors. Data are mean $\pm$ SD of 5 ($\alpha 1\beta 2$), 6 [$\alpha 1\beta 2\gamma 2$ (Q40X)], 6 [$\alpha 1\beta 2\gamma 2\gamma 2$ (Q40X)] and 8 ($\alpha 1\beta 2\gamma 2$) samples.

observed predominantly in the ER in the 20 remaining cells (18, 2, and 0).

For the heterozygous receptor, 47 cells (13, 22, and 12 in each of three independent replications) were counted in the short incubation condition, and 64 cells (19, 16, and 29) in the long incubation condition; all cells expressed the $\alpha 1^{myc}$ subunit. In the short incubation condition, the $\alpha 1^{myc}$ subunits were found predominantly on the membrane in 3 cells (2, 1, and 0 in each of the three replications), whereas the $\alpha 1^{myc}$ subunits were found predominantly in the ER in 44 cells (11, 21, and 12). In the long incubation condition, the $\alpha 1^{myc}$ subunits were found predominantly on the membrane in 34 cells (9, 9, and 16 in each of the three replications), whereas the $\alpha 1^{myc}$ subunits were observed dominantly in the ER in 30 cells (10, 7, and 13).

For the homozygous receptor, 114 cells (62, 15, and 37 in each of the three replications) were counted in the short incubation, and 137 cells (28, 47, and 62) in the long incubation; all cells expressed the $\alpha 1^{myc}$ subunit. In the short incubation, the $\alpha 1^{myc}$ subunits were found predominantly

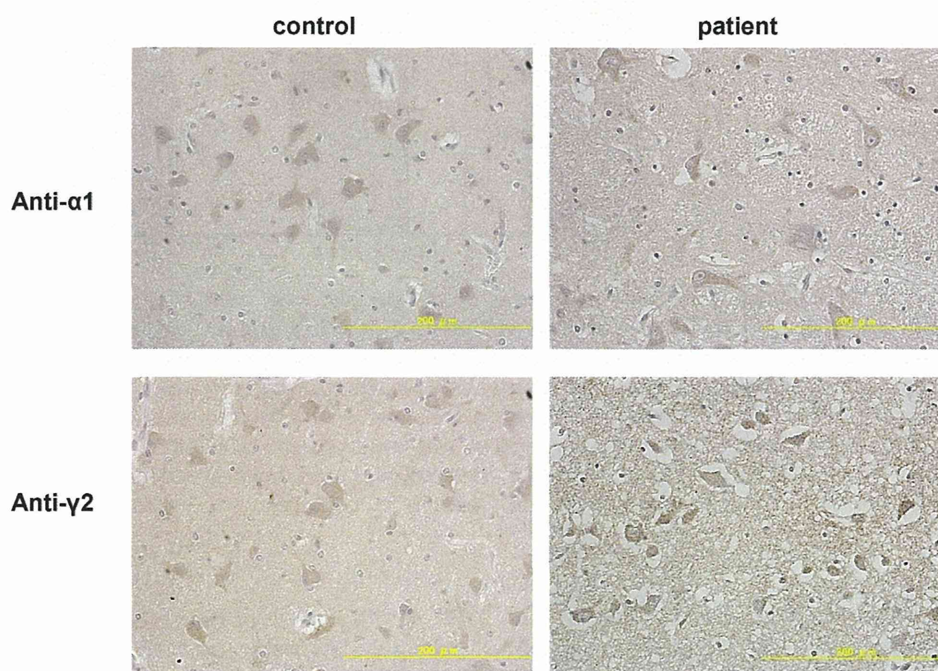

Figure 3 Immunohistochemistry of brain tissues. Immunostaining of brain tissue from one of the twins with antibodies against $\alpha 1$ and $\gamma 2$ subunits revealed partial loss of reactivity and granules in the somas of some neurons and neuropils. Magnification, 250×.

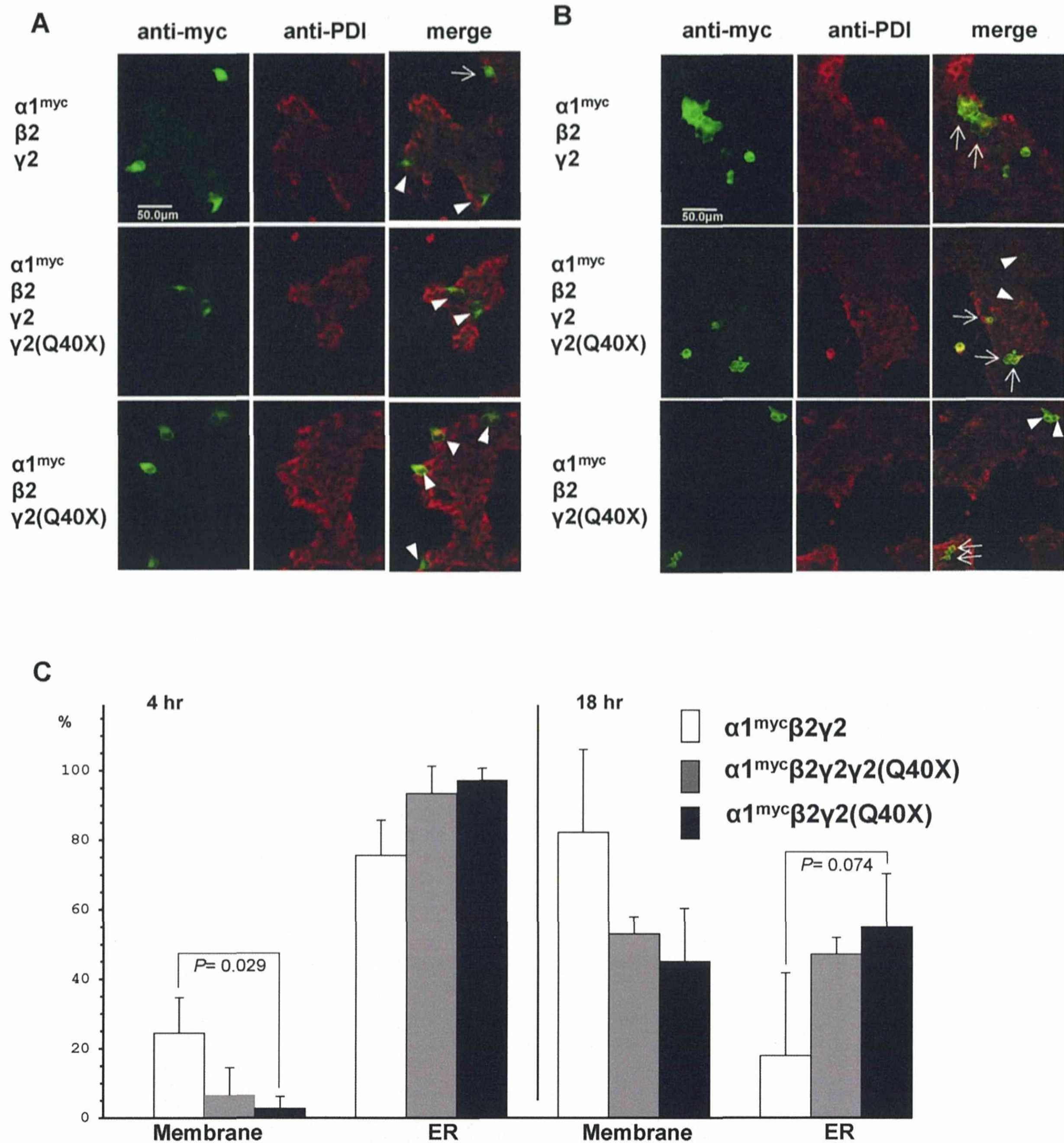

Figure 4 Localization of α1 subunits in HEK293T cells after short (4h) and long (18h) incubation periods. HEK293T cells were microinjected with wild type $\alpha 1^{myc}\beta 2\gamma 2$, heterozygous $\alpha 1^{myc}\beta 2\gamma 2\gamma 2$ (p.Q40X), and homozygous $\alpha 1^{myc}\beta 2\gamma 2$ (p.Q40X). After 4h or 18h harvest, cells were fixed and stained with antibodies for the myc-tag (green) and PDI (red). (A) The majority of wild type, heterozygous, and homozygous proteins colocalized with the ER (triangle). However, only some of the wild type also colocalized with the plasma membrane (arrow). (B) Approximately half of the heterozygously and homozygously expressed transcripts remained in the ER (triangle), while the majority of wild type protein colocalized with the membrane (arrow). (C) Quantitative analysis of the fractions of total wild type, heterozygous, and homozygous $GABA_A$ receptors that colocalized to the membrane and ER. Data are mean $\pm$ SD of the fractions of colocalization elucidated from three independent replications.

on the membrane in only 2 cells (1, 1, and 0 in each of the three replications), whereas the $\alpha 1^{myc}$ subunits were found predominantly in the ER in 112 cells (61, 14, and 37). Even in the long incubation condition, the $\alpha 1^{myc}$ subunits were found predominantly on the membrane in only 59 cells (13, 28, and 18 in each of the three replications), whereas the $\alpha 1^{myc}$ subunits were observed dominantly in the ER in 78 cells (15, 19, and 44).

Thus, in the short incubation condition, the average fraction of colocalization with the membrane for $\alpha 1^{myc}$ subunit

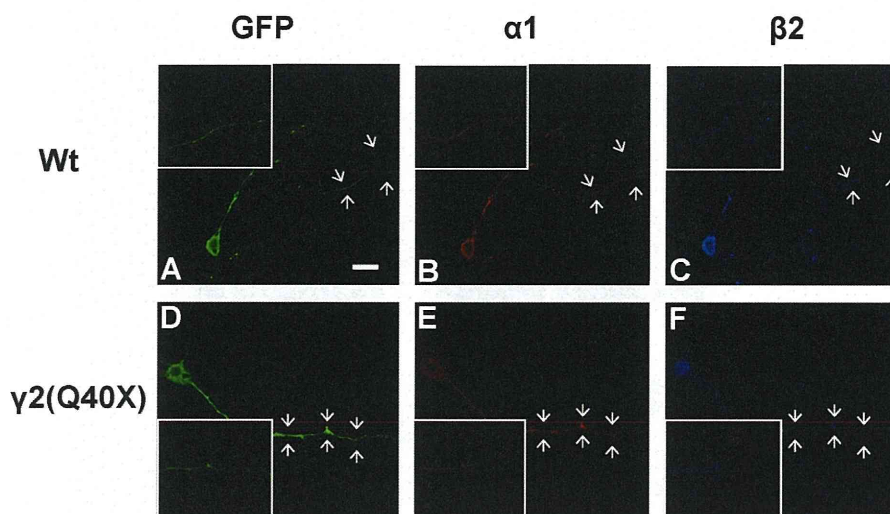

Figure 5 Axonal transport deficits of α1 and β2 subunits in neurons. Mouse hippocampal neurons were transfected with either wild type γ2 cDNAs or mutated (Q40X) cDNAs binding GFP after the IRES sequence. At 48 h after transfection, the cells were fixed, permeabilized, and stained with antibodies for GFP (green), α1 (red), and β2 (blue). Inserts are higher-magnification views of the position indicated by arrows. In neurons transfected with wild type γ2, GFP (A), α1 (B), and β2 (C), signals were strong in the axon terminals, while neurons expressing the mutated γ2 (Q40X), GFP (D), α1 (E), and β2 (F) showed lower staining intensity in their axons.

across the three replications was 24.4% for the WT receptor (26.7%, 33.3%, and 13.3% in each replication), 6.6% for the heterozygous receptors (15.4%, 4.5%, and 0%), and 2.8% for the homozygous receptor (1.6%, 6.7%, and 0%). Compared with the homozygous receptors, the WT receptors showed significantly higher colocalization with the membrane ($P = 0.029$) (Fig. 5C).

In the longer incubation condition, average fraction of colocalization with the membrane was 82% for the WT receptor (55.0%, 91.3%, and 100% in each replication), 52.9% for the heterozygous receptors (47.4%, 56.3%, and 55.2% in each replication), and 45.0% for the homozygous receptors (46.4%, 59.6% and 29.0% in each replication). Compared to the WT receptors, the homozygous receptors showed a trend to be retained in the ER ($P = 0.074$). Thus, it seems that mutant γ2 cDNAs interfered with the intracellular sorting of the receptor, resulting in its retention in the ER, even though this interference was evident only when cDNA of the mutant γ2 was injected with those of α1 and β2. These results suggest that this γ2 subunit harboring the p.Q40X mutation acts in a dominant-negative manner on intracellular trafficking of the $GABA_A$ receptor.

Axonal transport deficits of GABRA1 and GABRB2 proteins in neurons

In HEK cells, the $GABA_A$ receptor was located on the surface. To investigate whether the *GABRG2* mutant (p.Q40X) would prevent intracellular trafficking of endogenous α1 and γ2 subunits, we examined the axons of mouse hippocampal neurons in detail (Fig. 5). WT γ2 or mutated γ2 cDNA were transfected into cultured mouse hippocampal neurons and the transfected cells were identified by anti-GFP antibody. The intracellular distribution of both endogenous α1 and β2 subunits was examined by confocal microscopy after labeling with respective antibodies. The distributions of both endogenous α1 and β2 transfected mutated γ2 cDNAs were different from those of the transfected WT γ2 cDNA. In the axonal pathway, a significant portion of α1 and β2 proteins also bearing WT γ2 was distributed in the axon terminal. Interestingly, the signal intensity of both α1 and β2 subunits bearing mutant γ2 was reduced, and the signal of both subunits did not represent axon terminal. These findings suggested that the amount of transported α1 and β2 subunits was lower in neurons harboring the mutated γ2 allele compared to those with only the WT allele.

Dravet syndrome-associated *GABRG2* mutant

We next analyzed the effects of expression of the *GABRG2* mutant (p.Q40X) on the localization of α1 subunits in cultured mouse hippocampal neurons (Fig. 6). WT γ2 cDNAs transfected into mouse hippocampal neurons induced translocation of endogenous α1 subunits from the cell body to neuritic tip. In contrast, expression of the mutated γ2 (Q40X) cDNAs limited the endogenous α1 subunits to mainly the cell body. Moreover, the majority of the α1 subunits was detected on the surface of cell bodies, dendrites, and axons in neurons expressing WT γ2 cDNAs, while these units were absent at these locations in neurons expressing the mutated γ2 cDNAs. Moreover, α1 subunits partially colocalized with calnexin, another ER protein, but not with Golgi markers, GM130 and p115. However, statistical quantification regarding the distribution of the $GABA_A$ receptor in neurons could not be conducted for this study. Therefore, it cannot be definitely concluded that the γ2 subunit harboring the Q40X mutation interferes with the transportation of endogenous α1 subunits in neurons, as has been shown in the experiments with HEK cells.

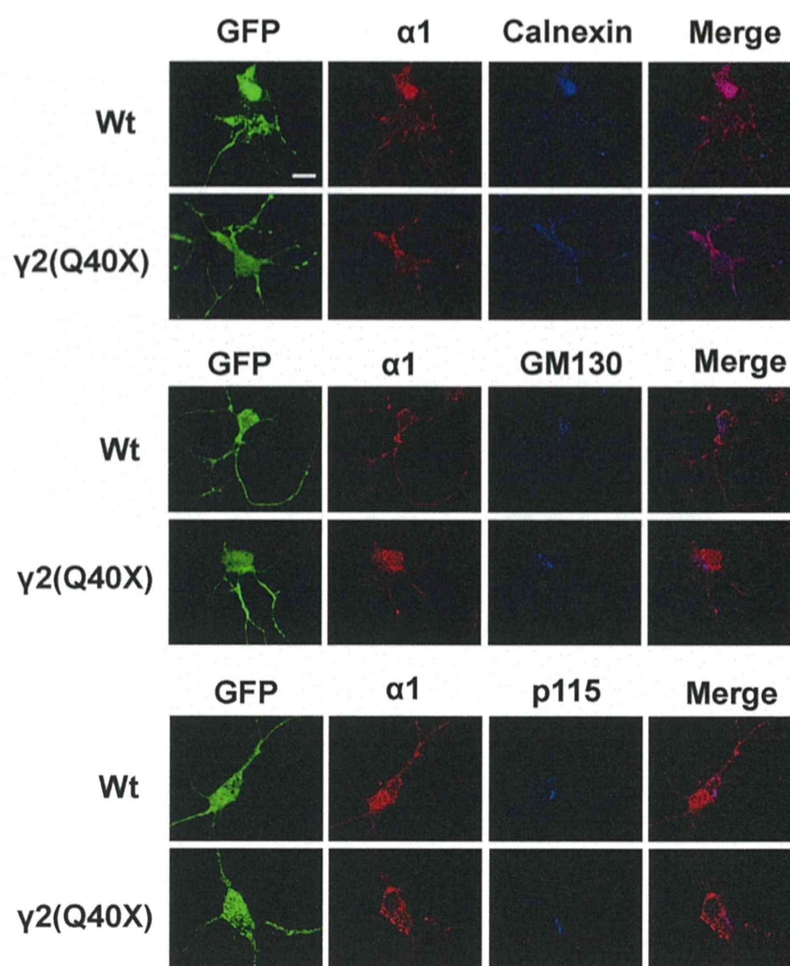

Figure 6 Accumulation of $\alpha 1$ and $\beta 2$ subunits in ER neurons. Mouse hippocampal neurons were transfected with either wild type $\gamma 2$ cDNAs or mutated (Q40X) cDNAs binding GFP after the IRES sequence. At 48 h after transfection, the cells were fixed, permeabilized, and immunostained for GFP (green) and $\alpha 1$ (red). An antibody to calnexin was used to mark ER (blue), while the Golgi apparatus was labeled using antibodies to GM130 and p115 (blue). In the presence of the mutated $\gamma 2$ (Q40X), endogenous $\alpha 1$ subunits were detected mainly in the cell body. The merged images show no overlap of the subunits with GM130 or p115.

Discussion

This study provided new insight into the pathogenesis of epilepsy by showing that a nonsense mutation of *GABRG2* found in a severe epilepsy phenotype results in abnormal intracellular trafficking of $GABA_A$ receptors. We encountered twin individuals with Dravet syndrome, who had a heterozygous nonsense mutation in *GABRG2* (p.Q40X). Interestingly, this mutation was also detected in the twins' father who was asymptomatic. Electrophysiological studies in HEK cells with reconstituted $GABA_A$ receptors showed reduced GABA-induced receptor currents when mutant $\gamma 2$(Q40X) protein was coexpressed with other components of the $GABA_A$ receptor, the $\alpha 1$ and $\beta 2$ subunits. Immunohistochemical studies demonstrated the presence of mutant-positive granules in neuronal soma and neuropils, while immunocytochemical studies to determine the localization of $\alpha 1$ and $\beta 2$ subunits showed disruption of intracellular trafficking of $GABA_A$ receptors with receptors retained in the ER following injection of mutated $\gamma 2$ cDNA into the HEK cells.

The first nonsense mutation of *GABRG2*, Q351X, was reported in a male individual in a large Austrian family where a number of affected individuals showed mainly simple FS and myoclonic astatic epilepsy (Harkin et al., 2002). The Q351X mutant was expressed heterozygously in an individual whose phenotype was consistent with Dravet syndrome in a family with GEFS+. This mutation is located in the intracellular loop between the third and fourth transmembrane domains of the $\gamma 2$ subunit and results in a truncated protein. Reconstituted $GABA_A$ receptor comprising WT $\alpha 1$ and $\beta 2$ subunits and the mutant $\gamma 2$ subunits in *Xenopus* oocytes showed no GABA-evoked currents. Retention of the mutant receptor in the ER was detected in HEK cells cotransfected with $\alpha 1$, $\beta 2$, and the mutant $\gamma 2$ subunits. The same mutation has been associated with both loss of function and dominant-negative suppression (Kang et al., 2009), and is located at the N-terminus of the molecule. Mutant transcripts may therefore undergo nonsense-mediated decay (NMD). A possible explanation of the molecular pathology associated with the p.Q40X mutation is haploinsufficiency caused by NMD.

However, the heterozygous *GABRG2* (+/-) gene deletion mice showed no convulsive seizures (Crestani et al., 1999). Heterozygosity of this nonsense mutation in the *GABRG2* gene in mouse may not have equivalent consequences in human, but the findings in mice raise the alternative possibility that the truncated protein caused by this mutation (p.Q40X) is actually produced and exerts a dominant-negative suppression effect on the function of the remaining WT $GABA_A$ receptors. Moreover, the proband's brain specimens immunostained against $\alpha1$ and $\gamma2$ subunits showed $GABA_A$ receptor harboring the mutant $\gamma2$ (Q40X) subunit and forming aggregates in cells of the brain. These results suggest that this mutation affects the construction and intracellular sorting of the $GABA_A$ receptor molecule.

$GABA_A$ receptors are assembled at the ER and then transported to the Golgi apparatus (Connolly et al., 1996; Gorrie et al., 1997), although the majority of $GABA_A$ receptors harboring the p.Q40X mutation colocalized within the ER. The $\gamma2$ subunits of the $GABA_A$ receptor are required for clustering of major postsynaptic $GABA_A$ receptor subtypes and trafficking of the intracellular $GABA_A$ receptor (Essrich et al., 1998; Korpi et al., 1994; Mu et al., 2002; Taylor et al., 1999, 2000). The p.Q40X mutation also interfered with the transport of $GABA_A$ receptors to the cell surface membrane.

Four mutations in *GABRG2* have thus far been associated with FS followed by childhood absence epilepsy (CAE) (Hales et al., 2005; Kang and Macdonald, 2004; Sancar and Czajkowski, 2004; Wallace et al., 2001), FS (Audenaert et al., 2006), GEFS+ (Baulac et al., 2001), and Dravet syndrome (Harkin et al., 2002). Screening for consistent electrophysiological patterns to segregate GEFS+ and Dravet syndrome have largely been unproductive (Kanai et al., 2004; Rhodes et al., 2004; Sugawara et al., 2003; Yamakawa, 2005). Although HEK293T cells transfected with mutated $\gamma2$ subunits (p.Q40X) produced GABA-induced current amplitudes of $GABA_A$ receptors, the proband was diagnosed as Dravet syndrome. We confirmed with the experiments on HEK cells that the majority of $GABA_A$ receptors harboring the p.Q40X mutation showed heterozygous and homozygous trafficking defect with respect to translocation of the receptor and its components to the surface of cell. Although statistical quantification could not be conducted, similar findings were observed in cultivated hippocampal neurons. Thus, $GABA_A$ receptors with the mutant $\gamma2$ subunit might be aggregated in the ER of neurons in the patients' brain. Although the proband's father harboring the same mutation was asymptomatic, the finding implicates a variable modifier, and strong candidates include factors related to the ER stress response. The association of apoptosis with certain neurological disorders was reported recently (Inoue et al., 2004; Khajavi et al., 2005), although the association of apoptosis with epilepsy has not yet been confirmed. Electrophysiological assays were performed on living cells in this study, and not on cells that had undergone apoptosis. Hence, studying the cells before any apoptotic events should allow the detection of GABA-induced current amplitudes. However, apoptotic neurons are often not found in vivo, thus explaining the appearance of phenotype as Dravet syndrome. Hence, the proband might actually have a Dravet syndrome phenotype.

In conclusion, our investigation of *GABRG2* mutation (Q40X) demonstrated the dominant-negative effects of $\alpha1$ and $\gamma2$ subunits. This result suggests that the differences among the phenotypes of epilepsy are likely due to the mutant $\gamma2$ subunit. This paradigm involves a channel trafficking abnormality followed by ER accumulation. Understanding the pathogenic mechanism could provide clues to address unsolved questions about epilepsy, such as the differences between GEFS+ and Dravet syndrome. These phenotypically different entities share the same genetic abnormalities and hence are generally considered to part of a single disease spectrum or function as allelic variants.

Conflict of interest

None of the authors has any conflict of interest to disclose.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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CHAPTER

Mutant $GABA_A$ receptor subunits in genetic (idiopathic) epilepsy

3

Shinichi Hirose[1]

Department of Pediatrics, School of Medicine, Fukuoka University, Fukuoka, Japan
Central Research Institute for the Molecular Pathomechanisms of Epilepsy, Fukuoka University, Fukuoka, Japan
[1]*Corresponding author: Tel.: +81-92-801-1011; Fax: +81-92-862-6955, e-mail address: hirose@fukuoka-u.ac.jp*

Abstract

The γ-aminobutyric acid receptor type A ($GABA_A$ receptor) is a ligand-gated chloride channel that mediates major inhibitory functions in the central nervous system. $GABA_A$ receptors function mainly as pentamers containing α, β, and either γ or δ subunits. A number of antiepileptic drugs have agonistic effects on $GABA_A$ receptors. Hence, dysfunctions of $GABA_A$ receptors have been postulated to play important roles in the etiology of epilepsy. In fact, mutations or genetic variations of the genes encoding the α1, α6, β2, β3, γ2, or δ subunits (*GABRA1*, *GABRA6*, *GABRB2*, *GABRB3*, *GABRG2*, and *GABRD*, respectively) have been associated with human epilepsy, both with and without febrile seizures. Epilepsy resulting from mutations is commonly one of following, genetic (idiopathic) generalized epilepsy (e.g., juvenile myoclonic epilepsy), childhood absence epilepsy, genetic epilepsy with febrile seizures, or Dravet syndrome. Recently, mutations of *GABRA1*, *GABRB2*, and *GABRB3* were associated with infantile spasms and Lennox–Gastaut syndrome. These mutations compromise hyperpolarization through $GABA_A$ receptors, which is believed to cause seizures. Interestingly, most of the insufficiencies are not caused by receptor gating abnormalities, but by complex mechanisms, including endoplasmic reticulum (ER)-associated degradation, nonsense-mediated mRNA decay, intracellular trafficking defects, and ER stress. Thus, $GABA_A$ receptor subunit mutations are now thought to participate in the pathomechanisms of epilepsy, and an improved understanding of these mutations should facilitate our understanding of epilepsy and the development of new therapies.

Keywords

apoptosis, channelopathy, convulsions, endoplasmic reticulum-associated degradation, ER stress, GABA, nonsense-mediated mRNA decay, severe myoclonic epilepsy in infancy, ubiquitin

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