The human epilepsy mutation **GABRG2(Q390X)** causes chronic subunit accumulation and neurodegeneration

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Genetic epilepsy and neurodegenerative diseases are two common neurological disorders that are conventionally viewed as being unrelated. A subset of patients with severe genetic epilepsies who have impaired development and often go on to die of their disease respond poorly to anticonvulsant drug therapy, suggesting a need for new therapeutic targets. Previously, we reported that multiple GABA<sub>A</sub> receptor mutations result in protein misfolding and abnormal receptor trafficking. We have now developed a model of a severe human genetic epileptic encephalopathy, the **Gabrg2<sup>+</sup>/Q390X** knock-in mouse. We found that, in addition to impairing inhibitory neurotransmission, mutant GABA<sub>A</sub> receptor 2(Q390X) subunits accumulated and aggregated intracellularly, activated caspase 3 and caused widespread, age-dependent neurodegeneration. These findings suggest that the fundamental protein metabolism and cellular consequences of the epilepsy-associated mutant 2(Q390X) ion channel subunit are not fundamentally different from those associated with neurodegeneration. Our results have far-reaching relevance for the identification of conserved pathological cascades and mechanism-based therapies that are shared between genetic epilepsies and neurodegenerative diseases.

Genetic epilepsies (GEs) are common neurological disorders that are frequently associated with ion channel gene mutations. Although many GEs are relatively benign, there is a group of GEs, the epileptic encephalopathies, which include Dravet syndrome, that are associated with intractable seizures, impaired development, severe cognitive impairment and sudden death. It is not known, however, whether the intractable seizures or some underlying progressive pathological processes are responsible for their clinical outcomes or whether the clinical features of the epileptic encephalopathies are caused by the impaired channel function, the presence of intracellular mutant ion channel protein or both.

The epileptic encephalopathy Dravet syndrome has been shown to be associated with mutations in sodium channel (SCN1A, SCN2A and SCN1B)²⁻⁵ and GABA<sub>A</sub> receptor (GABRG2 (refs. 6, 7) and GABRA1 (ref. 8)) subunit genes. **Gabrg2(Q390X)** is a mutation heterozygously associated with Dravet syndrome (note that the GABRG2(Q390X) mutation is also referred to as GABRG2(Q351X)⁷,⁹,¹⁰ when it does not include the 39 amino acid signal peptide). We have extensively characterized the cellular effects of this mutation in vitro⁹⁻¹¹, demonstrating that, similar to many other truncated proteins, γ2(Q390X) subunits are nonfunctional. In addition, however, the subunits accumulated in the endoplasmic reticulum (ER), where they produced dominant-negative suppression of the biogenesis and trafficking of wild-type subunits.¹⁰ The γ2(Q390X) subunits dimerized quickly once translated, accumulated and disturbed cellular hemostasis in a concentration-dependent manner.¹¹ It is well established that sustained ER stress leads to neurodegeneration.¹² However, the long-term cellular consequences of a misfolded mutant ion channel subunit associated with GE have not been addressed.

We generated a heterozygous Gabrg2<sup>+</sup>/Q390X knock-in (KI) mouse and compared it with a heterozygous Gabrg2<sup>+/−</sup> knockout (KO) mouse that has loss of functional γ2 subunits without accumulation of the mutant subunits. The Gabrg2<sup>+/−</sup> mouse has been reported to have mild hyperanxiety¹³ and to have brief absence seizures only in mice with the seizure prone DBA2J genetic background.¹⁴ In contrast, we found that Gabrg2<sup>+/Q390X</sup> mice had a severe epilepsy phenotype that included spontaneous generalized tonic-clonic seizures in mice with the seizure-resistant C57BL/6J background, suggesting that the mutation produces epilepsy by a mechanism(s) other than simple haploinsufficiency. Furthermore, we found that Gabrg2<sup>+/Q390X</sup> mice had increased mortality, reminiscent of sudden unexpected death in epilepsy (SUDEP) in humans. We compared the KI and KO mice functionally and biochemically and found that Gabrg2<sup>+/Q390X</sup> mice formed intraneuronal detergent-resistant, high molecular-mass protein complexes containing γ2 subunits and displayed widespread caspase 3 activation and sporadic neuronal death in the mouse brain, especially in the cortex, that increased in severity with aging. Our results provide evidence that an epilepsy ion channel gene mutation directly causes chronic neurodegeneration in vivo, and the presence of neurodegeneration, in addition to intractable seizures, provides a possible explanation, at least in part, for several key features of epileptic encephalopathies, including the bases for phenotypic severity, drug resistance, progressive course and poor outcome. Our findings suggest that the protein metabolism of an ion channel epilepsy mutation is not fundamentally different from that associated with neurodegeneration, and drugs developed to treat neurodegeneration might therefore be repurposed to treat severe genetic epilepsies by targeting the same mechanisms.

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Received 3 November 2014; accepted 22 April 2015; published online 25 May 2015; doi:10.1038/nn.4024
RESULTS

Mutant γ2(Q390X) subunits are aggregation prone

GABA_A receptors are the major mediators of inhibitory neurotransmission in the brain and are primarily composed of 2α, 2β, and 1γ subunits (Supplementary Fig. 1a). The GABRG2(Q390X) mutation was identified in two separate pedigrees with epilepsy, including Dravet syndrome 2, and produced a truncated subunit with the loss of 78 C-terminal amino acids (Supplementary Fig. 1b). We used protein structure modeling of the wild-type and mutant γ2 subunits without the extracellular N-terminal domain to predict the potential consequences on structure of the mutant subunit. For the mutant γ2(Q390X) subunit, the hydrophobic fourth transmembrane α-helix (YARIFFPTAFCLFNLVYWVSYLYL) was deleted and a new α-helix with many charged amino acids (KDKDKKKKNPAPTDIRPRSATI) was found to assume its location (Fig. 1a). The upper domain of the wild-type γ2 subunit was mainly hydrophobic and embedded in the membrane as a transmembrane domain, whereas the lower domain was hydrophilic and intracellular (Fig. 1a). The mutant γ2(Q390X) subunit had more hydrophilic surface area than the wild-type subunit (Fig. 1a) as a result of the new α-helix (KDKDKKKKNPAPTDIRPRSATI) that was found to be unstable in the membrane, thereby changing the subunit from a transmembrane protein to a globular cytosolic protein. The remaining hydrophobic surfaces in the α-helices of the mutant subunit were no longer in the membrane and were predicted to become ‘sticky’ in solvent, that is, the domains were predicted to bind to each other or to other proteins as a result of hydrophobic interactions. On the basis of a docking study for multiple interacting molecules, we determined that a number of relatively stable homodimer conformations might be formed as a result of nonspecific self-interactions of mutant γ2 subunits (Supplementary Fig. 1c).

**Figure 1** The GABRG2(Q390X) mutation associated with the epileptic encephalopathy Dravet syndrome caused the mutant subunit to be aggregate prone and to accumulate intracellularly. (a) Predicted structural models. Left and middle, protein surfaces of wild-type γ2 and mutant γ2(Q390X) subunits, respectively, where green indicates hydrophobic residues and red shows hydrophilic (polar or charged) residues. Right, the mutant γ2(Q390X) subunit, the hydrophobic fourth transmembrane α-helix (YARIFFPTAFCLFNLVYWVSYLYL) was deleted and a new α-helix with many charged amino acids (KDKDKKKKNPAPTDIRPRSATI) was found to assume its location (Fig. 1a). The upper domain of the wild-type γ2 subunit was mainly hydrophobic and embedded in the membrane as a transmembrane domain, whereas the lower domain was hydrophilic and intracellular (Fig. 1a). The mutant γ2(Q390X) subunit had more hydrophilic surface area than the wild-type subunit (Fig. 1a) as a result of the new α-helix (KDKDKKKKNPAPTDIRPRSATI) that was found to be unstable in the membrane, thereby changing the subunit from a transmembrane protein to a globular cytosolic protein. The remaining hydrophobic surfaces in the α-helices of the mutant subunit were no longer in the membrane and were predicted to become ‘sticky’ in solvent, that is, the domains were predicted to bind to each other or to other proteins as a result of hydrophobic interactions. On the basis of a docking study for multiple interacting molecules, we determined that a number of relatively stable homodimer conformations might be formed as a result of nonspecific self-interactions of mutant γ2 subunits (Supplementary Fig. 1c).
and a number of nonspecific hetero-dimer conformations might be formed between the mutant \( \gamma_2 \) subunit and its wild type–partnering \( \alpha_1 \) subunit (Supplementary Fig. 1d). All of these subunit dimers were predicted to be stable.

The \( GABRG2(Q390X) \) mutation promoted intraneuronal accumulation of mutant \( \gamma_2(Q390X) \) subunits in KI mice

To fully characterize the pathological basis for the epilepsy associated with the Q390X mutation, we generated the heterozygous \( GABRG2^{+/Q390X} \) KI mouse line with a standard gene targeting strategy (Fig. 1h). In both transfected HEK 293T cells and in brains from 1-week-old to 14-month-old \( GABRG2^{+/Q390X} \) mice, mutant \( \gamma_2(Q390X) \) subunits formed oligomers, likely dimers, tetramers and/or high molecular mass protein complexes on SDS-PAGE (Fig. 1c). The predicted molecular mass of the mutant \( \gamma_2(Q390X) \) subunit was 40 kDa, and the full-length wild-type \( \gamma_2 \) subunit was about 50 kDa (Fig. 1c). In HEK 293T and COS-7 cells cotransfected with \( \alpha_1, \beta_2 \) and \( \gamma_2(Q390X) \) subunits and in cultured rat cortical neurons transfected with \( \gamma_2 \) YFP or \( \gamma_2 \) YFP and \( \gamma_2(Q390X) \) YFP subunits alone, the mutant subunits accumulated intracellularly in the ER (Fig. 1d).

Immunohistochemical staining with antibody to \( \gamma_2 \) subunit consistently indicated that \( \gamma_2 \) subunits accumulated in the cortex of 2- and 16-month-old KI mice (Fig. 1e–g), as well as in postnatal day 0 (P0) KI mice and in multiple brain regions, including the cortex (Supplementary Fig. 2). The mutant \( \gamma_2 \) subunit fluorescence intensity was increased in cultured neurons (22.20 ± 0.97 versus 37.20 ± 2.5, \( n = 5 \) cultures, \( P = 0.0005 \), unpaired \( t \) test), as well as in both 2- and 16-month-old \( GABRG2^{+/Q390X} \) mice (Fig. 1h). In the brains of P0 mice, the \( \gamma_2 \) subunit staining was also stronger in \( GABRG2^{+/Q390X} \) than in wild-type mice, and in cerebellum, \( \gamma_2 \) subunit staining was prominent in the Purkinje cells layer, which contains Purkinje cells and Bergmann glia cells (Supplementary Fig. 2). The increased \( \gamma_2 \) subunits in the somatic regions in \( GABRG2^{+/Q390X} \) mice across different ages suggest that there was consistent ER retention of the mutant subunit protein.

\( GABRG2^{+/Q390X} \) KI mice had reduced viability

SUDEP is a substantial concern for patients with epilepsy, especially those with frequent and severe seizures, such as those associated with Dravet syndrome. SUDEP has been characterized in the \( Scn1a^{+/−} \) KO mouse model of Dravet syndrome, but the basis for SUDEP remains unclear. SUDEP could be a result of brain-driven cardiac dysfunction during seizures or to other non-cardiac mechanisms. Because the \( GABRG2(Q390X) \) mutation is associated with Dravet syndrome, we determined the mortality rate of heterozygous KI mice (Fig. 2). We genotyped all mice by PCR of tail DNA (Fig. 2a) and included all of the retrieved dead pups. We found that heterozygous and homozygous KI mice were born with a reduced Mendelian ratio (Fig. 2b). Homozygous \( GABRG2^{Q390X/Q390X} \) mice, however, did not survive beyond the day of birth (Fig. 2c), consistent with a previous report for homozygous \( GABRG2^{−/−} \) KO mice. However, gross morpholgy of heterozygous and homozygous KI mice (Fig. 2c), and the cellular structure of heterozygous KI pup's brains (Fig. 2d), appeared to be normal. Although viable, heterozygous KI mice had increased mortality throughout life (measured up to 30 weeks) and wild-type mice had minimal to no death over that time period (Fig. 2c). The increased death rate for heterozygous KI mice was likely related to severe generalized seizures, consistent with our observation that

Figure 2

\( GABRG2^{+/Q390X} \) KI mice had increased mortality both pre- and postnatally. (a) The wild-type \( \gamma_2 \) subunit allele PCR product was 323 bp and the mutant \( \gamma_2 \) subunit allele product was 405 bp as a result of the insertion of an 82-bp fragment, as shown in the PCR image for genotyping. (b) The distribution of each genotype for pups in mixed C57BL/6J/J29Swj (\( n = 7 \) litters, 58 pups) and C57BL/6J (\( n = 8 \) litters, 69 pups) backgrounds was plotted. (c) Mutant P0 pups were grossly normal. (d) Sections of cerebral cortex including the hippocampal formation were stained with hematoxylin and eosin. (e) A survival plot revealed the percentage of surviving mice with each genotype for 30 postnatal weeks (\( n = 51 \) for wild type and 47 for het). Note that homozygous KI mice did not survive beyond P0 (asterisk).

Figure 3

\( GABRG2^{+/Q390X} \) KI mice had severe seizures and behavioral comorbidities. (a) Representative EEG recordings show that the heterozygous (het) KI mice had interictal periods without (het baseline) or with (het ictal) epileptiform activity and had spontaneous generalized tonic-clonic seizures with epileptiform discharges (het GTCS). The top scale bars refer to traces 1–3, and the lower scale bars refer to traces 4 and 5. (b, c) KI mice had lowered seizure threshold with intraperitoneal PTZ administration (50 mg per kg). After PTZ injection, KI mice in the C57BL/6J background (open circles) progressed more rapidly to clonic seizures (b) and hind limb extension (c) than wild-type (WT) mice (filled circles) (\( n = 20 \) mice for wild type and 23 for het).
several mice died after the occurrence of spontaneous generalized tonic-clonic seizures during routine handling.

**Gabrg2**<sup>+/Q390X</sup> KI mice had spontaneous seizures and neurobehavioral comorbidities

We observed spontaneous generalized tonic-clonic seizures in Gabrg2<sup>+/Q390X</sup> mice at 19 d of age (Supplementary Video 1). To characterize the seizure phenotype of heterozygous KI mice, we recorded synchronized video electroencephalograms (EEGs) in mice. The EEGs demonstrated occurrence of spontaneous electrographic and behavioral seizures as well as interictal discharges (Fig. 3a and Supplementary Fig. 3). With blockade of GABA<sub>A</sub> receptors by pentylenetetrazol (PTZ, 50 mg per kg of body weight), heterozygous KI mice had lowered seizure threshold than wild-type littermates, as demonstrated by early onset and increased percentage of clonic seizures and tonic hind limb extension (Fig. 3b,c). We chose the lower dose of PTZ because of the high mortality rate of Gabrg2<sup>+/Q390X</sup> mice at higher doses of PTZ (70 mg per kg or 85 mg per kg).

We then assessed potential behavioral comorbidities of epilepsy in Gabrg2<sup>+/Q390X</sup> mice. We performed the elevated zero maze (EZM) test and the open field (OF) test. In the EZM test, heterozygous KI mice spent substantially less time in the open arm (121.6 ± 6.46 s versus 94.5 ± 3.67 s, n = 59 for wild type and 57 for heterozygous KI, unpaired t test) and had reduced total entries into the open arm (16.24 ± 0.91 versus 13.39 ± 0.61, n = 59 for wild type and 57 for heterozygous KI, unpaired t test) than wild types. In the OF test, KI mice had spontaneous seizures and SUDEP, enhanced anxiety and deficits in social behaviors, whereas Gabrg2<sup>+/−</sup> KO mice have been reported to be seizure free<sup>13</sup> or to have absence epilepsy only in a specific seizure-prone genetic background<sup>14</sup>.

**GABAergic mIPSC amplitude and frequency were reduced more in Gabrg2<sup>+/Q390X</sup> KI mice than in Gabrg2<sup>+/−</sup> KO mice**

Gabrg2<sup>+/−</sup> KI mice had severe epilepsy, increased mortality and neuropsychiatric comorbidities. By contrast, Gabrg2<sup>+/−</sup> KO mice were heterozygous KI mice had increased total distance traveled in the first 10 min (1,245.4 ± 31.2 cm versus 1,497.4 ± 54.1 cm, n = 35 for wild type and 47 for heterozygous KI, unpaired t test), but tended to be quiet and stayed in the corner of the chamber in the later time segments and had reduced time spent in the center during the 1-h test (31.28 ± 1.11 min versus 19.77 ± 1.01 min, n = 35 for wild type and 47 for heterozygous, P < 0.0001, unpaired t test). This was consistent with the fact that the heterozygous KI mice spent less time in the center resting (42.98 ± 2.58 s versus 33.72 ± 2.42 s, n = 35 for wild type and 47 for heterozygous KI mice, P = 0.012, unpaired t test) and more time in the periphery resting (81.06 ± 6.33 s versus 106.4 ± 5.82 s, n = 35 for wild type and 47 for heterozygous KI mice, P = 0.045, unpaired t test) per 5-min segment. Gabrg2<sup>+/Q390X</sup> KI mice had multiple phenotypes, including spontaneous seizures, SUDEP, enhanced anxiety and deficits in social behaviors, whereas Gabrg2<sup>+/−</sup> KO mice have been reported to be seizure free<sup>13</sup> or to have absence epilepsy only in a specific seizure-prone genetic background<sup>14</sup>.

**Figure 4** The GABAergic mIPSCs from Gabrg2<sup>+/Q390X</sup> KI mice were not equivalent to those from Gabrg2<sup>+/−</sup> KO mice. (a,b) Representative traces of GABAergic mIPSCs from cortical layer VI pyramidal neurons from two month old wild-type (WT) and heterozygous (het) KI (a) or KO (b) mice. (c,d) mIPSC amplitudes were plotted as a function of frequency of occurrence and amplitude (insets) and in cumulative histograms for both het KI (c) and KO (d) mice (for KI mice, n = 15 cells from 11 slices from 10 mice for wild type and 11 cells from 8 slices from 8 mice for het, unpaired t test, P = 0.022, wild type versus het; For KO mice, n = 8 cells from 6 slices from 5 mice for wild type, n = 9 cells from 7 slices from 6 het mice, P = 0.066, wild type versus het). Black line indicates wild type, gray line indicates heterozygous. Error bars represent s.e.m. *P < 0.01 versus wild type.

**Figure 5** Gabrg2<sup>+/Q390X</sup> KI mice were not equivalent to Gabrg2<sup>+/−</sup> KO mice with respect to the remaining wild-type GABA<sub>A</sub> receptor subunit expression. (a–d) Lysates from different brain regions (cortex (ctx), cerebellum (cb), hippocampus (hip) and thalamus (thal)) from heterozygous (het) KO and KI mice were subjected to SDS-PAGE and immunoblotted with antibody to γ2 subunit (a,b) or α1 subunit (c,d). (e–h) Integrated density values (IDVs) for total γ2 and α1 subunits from wild-type and het KI (e,g) or wild-type and het KO (f,h) mice were normalized to the Na<sup>+</sup>/K<sup>+</sup> ATPase or anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) loading control (LC) in each specific brain region and plotted. *P < 0.05, **P < 0.01, ***P < 0.001 versus wild type. n = 5 pairs of mice for KI and 5 pairs of mice for KO. Error bars represent s.e.m. Full-length gels are presented in Supplementary Figure 4.
Younger Gabrg2+/Q390X KO mice had increased γ2 subunit accumulation in neuronal somata, but reduced expression of γ2 subunits in synaptosomes and on the cell surface. (a) The brains from 6-month-old wild-type and heterozygous (het) KI littermates were blocked, short fixed with 4% paraformaldehyde for 30 min and immersed in 30% sucrose overnight. The brain tissues were sectioned by cryostat at 15 μm and stained with rabbit antibody to γ2 subunit (green), mouse antibody to gephyrin (red) and the cellular nucleus marker To-pro-3 (blue). The presented images are from cortex layers V-VI. (b) An enlarged image from the wild-type overlay was used to illustrate the quantification of the fluorescent intensity values in somatic and non-somatic regions with ImageJ. The fluorescent intensities of the whole field, somatic or non-somatic regions were measured. (c) The fluorescence intensity values in the nuclei were used as background values that were subtracted in each condition. The total fluorescence intensity values from the whole field and non-somatic regions were reduced, but were increased in the somata in the het mouse brains (P = 0.0048 for whole field, P = 0.0031 for somata, P = 0.0136 for nonsomata, n = 11 wild-type mice and 12 het mice, unpaired t test). (d,f) The forebrains of 2–4-month-old mice were subfractionated. Equal amounts of protein from nuclei and cell debris (p1), total cytosol (s1), cytosol and light membrane (s2), and synaptosomes (spm) were analyzed by SDS-PAGE and immunoblotted with antibody to γ2 subunit. The protein IDVs in each fraction were normalized to their own loading control (LC) Na+-K+ ATPase or β-Tubulin (P = 0.034 wild type versus het spm, n = 4 pairs of mice, 4 independent experiments, unpaired t test). (e,g) The surface proteins from the live mouse brain slices were biotinylated and analyzed by SDS-PAGE and immunoblotted with antibody to γ2 subunit (P = 0.011 for cor, P = 0.0088 for cb, P = 0.0154 for hip, P = 0.0007 for thal, n = 4 pairs of mice; one-sample t test). *P < 0.05, **P < 0.01, ***P < 0.001 versus wild type. Error bars represent s.e.m. Full-length gels for d and e are presented in Supplementary Figure 4.
surface (Fig. 6e–g and Supplementary Fig. 4). In summary, our data suggest that γ2 subunits in synapses were reduced in spite of their accumulation in the neuronal somatic region.

Older mutant Gabrg2γ2/Q390X KI mice had increased caspase 3 activation and cell death in the cerebral cortex

Although no in vivo evidence for neurodegeneration in genetic epilepsy has been reported previously, it has been established in multiple neurodegenerative diseases that sustained accumulation and aggregation of mutant protein will eventually lead to neurodegeneration.23–26 Thus, given that the mutant γ2(Q390X) subunit is aggregation prone and relatively stable, its sustained expression could lead to neurodegeneration. To examine neuronal apoptosis in heterozygous KI mice, we stained for γ2 subunit and cleaved caspase 3 in 1-year-old mice (Fig. 7). Cleaved caspase 3 has been used as a marker for cellular apoptosis because of its role in executing cell death.27 In Gabrg2γ2/Q390X KI mice, but not wild-type mice, increased cleaved caspase 3 staining and γ2 subunit accumulation were both present in the deep layers of the cortex (Fig. 7a,b). The intensity of both γ2 subunit and cleaved caspase 3 staining was higher in the neuronal somata of mutant than in wild-type mice (Fig. 7f). Cleaved caspase 3 was mainly present in the neuronal cytoplasm (Fig. 7c) and colocalized with γ2 subunits in what appeared to be aggregates (Fig. 7d). These cleaved caspase 3–γ2 subunit aggregates were absent in the old wild-type and young (2 month) KI mice. They were detectable in the 5–6-month-old KI mice, but were prominent in old (1 year or older) KI mice with neuronal loss in the cortex (Supplementary Figs. 5–8). We used paraffin-embedded brain tissues because the staining was focused on neuronal somatic regions. Synaptic proteins were poorly preserved in these tissues compared with those with short fixation (Figs. 1f,g and 6a,b and Supplementary Fig. 9).

We also asked whether the mutant γ2 subunit aggregates were associated with neuronal death. To do this, we performed TUNEL staining and found that the percentage of TUNEL-positive neurons in cortical layer IV–VI neurons was higher in 1-year-old heterozygous KI mice than in age-matched wild-type mice (Fig. 7e,g). It is believed that age is a risk factor for neurodegeneration and protein aggregation, and this concept has been well studied in neurodegenerative diseases that are caused by both genetic and environmental factors.28–31 Our data are consistent with studies of neurodegeneration; the older heterozygous mice had cleaved caspase 3 activation, cytoplasmic caspase 3–γ2 subunit–positive aggregates and neuronal death, whereas these features were not prominent in the younger Gabrg2γ2/Q390X mice (Supplementary Figs. 5–8 and 10–12).

The activation of caspase 3 could be a result of the accumulation of mutant γ2(Q390X) subunits independent of seizure activity

Although caspase 3 activation is a crucial event for neuronal death and an established feature of many neurodegenerative diseases,32 caspase 3 activation can be caused by other neuronal activities.33 We therefore examined the level of caspase 3 activation in cultured neurons in which no seizure activity was present. We found that more neurons were caspase 3 positive in those cultured from the heterozygous Gabrg2γ2/Q390X KO mice than from the wild-type mice (4.6 ± 1.7% versus 29.4 ± 6.7%, n = 4 cultures, P = 0.015, unpaired t test; Fig. 8a). In HEK 293T cells, we expressed wild-type γ2 or mutant γ2(Q390X) subunits with progressively increasing levels of cDNA and found that the cells expressing mutant γ2(Q390X) subunits

![Figure 7](image-url)

**Figure 7** Older Gabrg2γ2/Q390X KI mice had caspase 3 activation and neuronal death in the deep layers of cerebral cortex. (a,b) Brain sections from 1-year-old wild-type (WT) and heterozygous (Het) KI mice were stained with rabbit antibody to γ2 subunit (a) or rabbit antibody to cleaved caspase 3 (b). (c–e) Similar sections were stained for cleaved caspase 3 (green) in combination with mouse monoclonal antibody to neuronal marker NeuN (red) and cellular nuclei marker To-pro-3 (blue) (c), mouse antibody to γ2 subunit (red) and cellular nuclei marker To-pro-3 (blue) (d), or NeuN (red) and cell death marker TUNEL (green) (e). Arrows indicate areas shown at higher magnification (a,b,c,e). (f) The γ2 subunit and cleaved caspase 3 protein density values in the cell somata were quantified using ImageJ (P = 0.005 for γ2 subunit, P = 0.0161 for caspase 3 intensity, n = 12 sections from 7 mice for wild type and n = 11 sections from 7 mice for het, unpaired t test). *P < 0.05, **P < 0.01 versus wild type (n = 12 for wild type and 11 for het). (g) The cleaved caspase 3–positive staining cells in the total cellular population, the cleaved caspase 3–positive staining in NeuN-positive cells and the TUNEL-positive cells in the NeuN-expressing populations were quantified and plotted (P = 0.0301 for caspase 3, P < 0.0001 for caspase 3 + NeuN, P < 0.0001 for TUNEL, n = 7 pairs of mice, unpaired t test). *P < 0.05 versus wild type, ***P < 0.001 versus wild type. Error bars represent s.e.m. Scale bars in insets: a, 2 μm; b, 3 μm; c, 5 μm; e, 2.5 μm.
DISCUSSION

Dravet syndrome is an epileptic encephalopathy in which “the epileptic activity itself may contribute to severe cognitive and behavioral impairments above and beyond what might be expected from the underlying pathology alone (for example, cortical malformation), and that these can worsen over time” 14. Our results suggest that, in addition to the disinhibition produced by substantial loss of functional γ2 subunits leading to epilepsy, the progressive behavioral changes and worsening epilepsy caused by the GABRG2(Q390X) mutation may also be a result of neuronal dysfunction and neurodegeneration caused by the accumulation and aggregation of a truncated ion channel protein that increases cell stress and leads to chronic neurodegeneration.

There are no reports that the mutant protein associated with Dravet syndrome causes neurodegeneration. However, although not definitive, there are several lines of evidence that neurodegeneration may occur in a subset of patients with GE. For example, a recent study of a brain specimen from a Dravet syndrome patient harboring the GABRG2(Q40X) mutation indicated that the mutant γ2 subunits formed protein aggregates, but the details of the aggregates are not available6. Our in vitro study revealed that mutant γ2(Q390X) subunits produced substantial protein aggregates, whereas mutant γ2(Q40X) subunits were undetectable (Supplementary Fig. 13). In addition, a study using brain magnetic resonance imaging (MRI) in a large group of GE patients 35 found substantial cortical atrophy, suggesting possible neuronal loss. A brain morphometry study in Dravet syndrome patients revealed global volume reductions of gray and white matter, as well as a correlation between the intracranial volume reduction and increased age 16. The lack of clear clinical evidence of an association of mutations in GE and neurodegeneration may be a result of epileptic encephalopathies that occur in only a small subset of epilepsy patients, and the relationship may have gone unrecognized. In addition, the major cause of Dravet syndrome is SCN1A mutations, which may have a different pathophysiologic that GABRG2 mutations.

In Dravet syndrome, altered neurotransmission and seizure phenotypes have been extensively characterized in an Scn1a γ−/− KO mouse as well as in a nonsense mutation Scn1a KI mice 36,37. However, no mutant protein was detected in Scn1a KI mice, suggesting haploinsufficiency. We found that truncated γ2(Q390X) subunits were produced, that they accumulated in neurons and that, in older Gabrg2+/Q390X KI mice, they formed sporadic protein aggregates in multiple brain regions. These findings are consistent with the widespread expression of γ2 subunits in the brain 39. We previously reported that mutant γ2(Q390X) subunits produced dominant-negative suppression of wild-type–partnering and wild-type γ2 subunits 10, and had slow degradation and disturbed cellular homeostasis 39. Our findings suggest that an ion channel mutation associated with a severe GE results in activated caspase 3, protein aggregates and chronic neurodegeneration.

Gabrg2+/Q390X KI mice phenocopy the major symptoms of the human epileptic encephalopathy Dravet syndrome and the phenotypes of patients that heterozygously carry the mutation (Online Methods). Gabrg2−/+Q390X KI mice had multiple forms of epilepsy, including generalized tonic-clonic seizures and neuropsychiatric comorbidities, such as increased anxiety and social deficits and SUDEP. However, we did not find a difference in motor coordination between control and mutant mice up to 6 months of age, as determined by the rotarod test. This is in contrast with the findings that there are severe ataxia in...
heterozygous Scn1a-/- KO mice at P21 (ref. 40). This suggests that there may be differences in the comorbidities between the severe epilepsies associated with an SCN1A mutation and a GABRG2 mutation.

Comparison of Gabrg2+/Q390X KO and Gabrg2+/- KO mice revealed that GABAergic synaptic transmission in KI mice was not equivalent in wild-type GABAA receptor subunit biogenesis. Gabrg2+/- KO mice only had a small reduction in γ2 subunit expression, whereas α1 subunit expression was unchanged. In contrast, total expression of both α1 subunits and the remaining wild-type expression was unchanged. In addition, total expression of both γ1 subunits and the remaining wild-type γ2 subunits in Gabrg2+/-Q390X mice was reduced in all of the major brain regions, including cerebral cortex, cerebellum, hippocampus and thalamus, compared with wild-type littermates and Gabrg2+/- KO mice. This dominant-negative suppression of the wild-type subunits by γ2(Q390X) subunits is likely a result of oligomerization of mutant γ2(Q390X) subunits with wild-type subunits, thereby trapping the wild-type subunits in the ER. This likely explains, in part, why Gabrg2+/-Q390X KI mice had a more severe epilepsy phenotype.

Our results challenge the widely held belief that neurodegeneration does not occur in GEs. Much effort has been directed towards identifying neuronal death in different animal models of epilepsy, such as pilocarpine, kainic acid kindling and thermal animal models. However, these are models of acquired, rather than genetic, epilepsies. The cellular environment in Gabrg2+/-Q390X mice is different from that in the external stimulus-induced seizure models because the mutation is present from conception. We found that the KI mice had intraneuronal γ2 subunit–caspase 3 protein aggregates and had caspase 3 activation and chronic neurodegeneration, although these features were not present in younger mice expressing the mutant subunit. The reduction of neuronal number was 15% in the somatosensory cortex in the old KI mice, as indicated by neuronal marker NeuN staining. The reduction of neuronal number was 15% in the somatosensory cortex in the old KI mice, as indicated by neuronal marker NeuN staining. However, sporadic neuronal death was observed in multiple brain regions, including cortex, hippocampus, thalamus and cerebellum. In a given neuron, its viability and caspase 3 activation were likely correlated with the abundance of γ2(Q390X) subunits. We previously demonstrated that ER-retained trafficking-deficient γ2 subunits produce ER stress. It is possible that the activated caspase 3 also alters synaptic activity, as caspase 3 is required for the refinement of neuronal circuits during development that involves selective elimination of axons, dendrites and synaptic connections. However, it should be noted that, although mutant γ2 subunits accumulated in the neurons of mice at all ages, neuronal death in the KI mice was highly variable, chronic and subject to substantial developmental effect, as we found that neuronal death was only evident in the older KI mice. The identification of mutant protein aggregation in Gabrg2+/-Q390X KI mice has far reaching implications for both disease classifications and therapeutic strategies. Although GE and neurodegenerative diseases are two conventionally unrelated disease entities, our results suggest that the mutant protein metabolism and the engaged signaling pathways, such as caspase 3 activation, may be similar in both disorders. Thus, the therapeutic developments in neurodegeneration and other diseases with similar mechanisms could be repurposed to treat epilepsy by targeting the same signaling pathways.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

The authors would like to thank the Vanderbilt Translational Pathology core for tissue preparation and immunostaining, and K. Boyd for consultation on immunohistochemistry. We thank K.M. Verdier and A. Pimenta for animal husbandry, Z. Liu and J. Allison for assistance in neurobehavioral tests, and S. Zeng for computational assistance, as well as S. Joshi and J. Kapur (University of Virginia School of Medicine) for providing mouse monoclonal γ2 antibody, L. Wang (Vanderbilt University) for providing consultation on statistics, and T. Abel (Vanderbilt University) for consultation on stereology and cell counting. This work was supported by grants from Citizen United for Research in Epilepsy, the Dravet Syndrome Foundation and Dravet.org (which was previously named IDEAleague), a Vanderbilt Clinical and Translation Science Award to J.-Q.K., grants from the National Institute of Neurological Disorders and Stroke (R01 NS082635 to J.-Q.K., R01 NS51590 to R.L.M. and R01 GM10701 to D.X.), and a grant from the National Institute of Child Health and Human Development (P30HD15052) to Vanderbilt Kennedy Center.

AUTHOR CONTRIBUTIONS

J.-Q.K. and R.L.M. conceived the project. J.-Q.K. designed and performed experiments, analyzed the data, coordinated the study, supervised the project, and wrote the manuscript. R.L.M. supervised the animal breeding to develop the congenic wild-type and mutant strains. W.S. collected the biochemical data and helped with colony maintenance, genotyping and tissue preparation. C.Z. collected and analyzed the electrophysiology data. D.X. performed the protein modeling and wrote the protein modeling part of the paper. R.L.M. supervised the project, critically reviewed the data and edited the manuscript. All of the authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

The natural history of Dravet syndrome patients with the Gabrg2(Q390X) mutation. The Gabrg2(Q390X) mutation is associated with a small pedigree of Dravet syndrome as reported previously7.

The history of the patient in Harkin’s report has been mentioned in two papers8,9. In Harkin’s paper7, the patient’s history was documented as “The patient had had a severe phenotype of GEFS+, which was initially regarded as MAE, but further history showed she had SMEL. She had multiple clonic seizures affecting the right upper limb, associated with fever at 3 months of age. She had many such focal febrile seizures, as well as afebrile GTCS. But she did not have convulsive status epilepticus. Absence, myoclonic and tonic seizures began at 3–4 years of age. Myoclonus involving both upper limbs was prominent on examination and was exacerbated with lamotrigine. From 17 years of age, she had continuous irregular myoclonus of her right upper limb; by 22 years of age, she had 1–2 GTCS per year and 1–2 absence seizures. EEs became more active over time and showed frequent paroxysms of irregular generalized polyspike-wave activity brought out by photic stimulation. MRI was normal. Early motor developmental milestones were normal, but speech was delayed. Neuropsychological assessment at 14 years of age placed her in the moderately intellectually disabled range, without lateralizing features.”

In Jansen’s paper10, the patient was reported when she was 26 years old. Her age of seizure onset was 3 months. She first had febrile seizures, then she had TCS, myoclonic seizures (MS), absence seizures (AS), complex partial seizures (CPS) and tonic seizures (TS). Her diagnosis is SMEL. She had TCS, CPS and AS when she was 26 years old. Motor signs were not present. In Jansen’s paper, MRI scans were available in ten patients. Unilateral hippocampal atrophy was noted in two, generalized atrophy in two and six were normal. However, the authors did not specify which mutation had brain atrophy. To our knowledge, this is the only study that reports the natural history of Dravet syndrome with the Gabrg2(Q390X) mutation.

Mice. The Gabrg2+/Q390X KI mouse was generated in collaboration with R.L.M.'s laboratory and S.-P. Yee (University Connecticut Health Center). Briefly, genomic DNA containing Gabrg2 was retrieved from the BAC, RP23-2331N1, which was generated using DNA isolated from C57BL/6 mice. The targeting vector was prepared by recombining as described41. The targeting vector containing the 5′ long arm, which included exon 8, was approximately 5 kb followed by the loxP-PGK-neo-loxP cassette and exon 9 with the Q390X point mutation, which comprised the 3-kb 3′ short arm. A DraI restriction endonuclease site was engineered into the mutant Gabrg2 allele to help distinguish the mutant from the wild-type allele. Targeted mice were bred with Hprt-Cre mice to remove the PGK-neo cassette and screened by the loxP-PGK-neo-loxP cassette and exon 9 with the Q390X point mutation, which comprised the 3-kb 3′ short arm. A DraI restriction endonuclease site was engineered into the mutant Gabrg2 allele to help distinguish the mutant from the wild-type allele. Targeted mice were bred with Hprt-Cre mice to remove the PGK-neo cassette to generate the mutant KI mouse. The targeting vector containing the 5′ arm, which includes exon 8, is approximately 5 kb followed by the loxP-PGK-neo-loxP cassette and exon 9 with the Q390X (C1165T) point mutation, which comprise the 7-kb 3′ arm. The vector was linearized by NotI digestion before electroporation into embryonic stem (ES) cells, which was derived from F1 (C57BL/6/129Sv) embryo. After electroporation into ES cells and G418 and Ganc selection, the targeted ES clones were identified by using long range PCR followed by PCR/sequencing to confirm the presence of the C1165T point mutation. Chimera mice were generated by morula<→ES cell aggregation and then bred with Hprt-Cre mice to remove the PGK-neo cassette and screened for germline transmission.

The primer pairs flanking the loxP site for genotyping were gtf 5′-ATG GCCATGGAAGTGTGACA and gtr: 5′-TGATGGTCTCATGCTGCTTCT. The PCR fragment for the wild-type allele was 523 bp and the fragment for the mutant allele was 405 bp. The Gabrg2+/Q390X KO mouse line was provided by B. Luscher (Penn State University)42. The mice used for biochemistry, immunohistochemistry and electrophysiology studies were from mixed C57BL/6/129Sv background. The mice used for EEG recordings and neurobehavioral experiments were congenic lines and were bred into C57BL/6 mice for at least eight generations. All behavioral tests were done blind to mouse genotype with age matched littermates. All the EEG surgery and recordings as well as behavioral tests were approved by Institutional Animal Care and Use Committee at Vanderbilt University.

There was no sharing of wild-type mice between KI and KO lines. The wild-type and mutant mice were paired before the experiments. All of the experiments were run in parallel. Each pair of wild-type and mutant KI or KO mice were killed at the same time, the samples were prepared at the same time, samples from the four brain regions (cortex, cerebellum, hippocampus and thalamus) of each pair were run at the same time in the same gel. Each data point for wild-type and mutant mice were from the same membrane, and the protein of interest was normalized to the loading control Na+/K+-ATPase or GAPDH band from the same membrane each time.

Plasmid and antibodies. The human γ2(Q390X) and γ2(Q390X)YFP subunit plasmids were described in a previous study10. Rabbit polyclonal anti-γ2 subunit antibody (224-003, 1:300) was purchased from Synaptic System. Mouse monoclonal anti-γ2 subunit antibody used in Figure 7d was a gift provided by S. Joshi and J. Kapur (University of Virginia, Health Sciences Center) (1:50). The specificity of this antibody has been validated by J. Kapur’s laboratory, and the antibody is commercially available44. Mouse monoclonal anti-α1(N95/35) (1:1,000) was from the University of California Davis/National Institutes of Health NeuroMab Facility. Rabbit anti-cleaved caspase 3 (96645, 1:300) was from Cell Signaling Technology. The mouse monoclonal anti-neuronal nuclei (NeuN) antibody was from Millipore (MAB377, 1:100) or from Abcam (ab104224, 1:100). The rabbit polyclonal anti-Er81 (AB10554, 1:100) and Trbl (ab10806) antibodies were from Millipore or Abcam. The mouse monoclonal GAD153 (AB11419, 1:100) antibody was purchased from Abcam. The rabbit anti-calbinbin D-28K antibody (CB38, 1:200) was from Swant. For protein loading internal controls, mouse monoclonal anti-Na+ /K+ ATPase α subunit (aef; 1:1,000) was from the Developmental Studies Hybridoma Bank, and the rabbit polyclonal GAPDH antibody (AB9485, 1:2,000) was from Abcam. Mouse monoclonal β-Tubulin antibody (TB328, 1:500) was from Sigma. The fluorescently conjugated goat anti-rabbit-680 (926-32221) and goat anti mouse-800 (926-32210) secondary antibodies were from LI-Cor (1:10,000).

Brain slice immunohistochemistry. We used brain sections both with the conventional paraffin embedding method and with the short fixation protocol to preserve the postsynaptic proteins45. TUNEL staining was performed with the TACS 2 TdT-Fluor in situ apoptosis detection kit from Trevigen following the manufacturer’s instructions and as performed in a previous study46,47.

For freshly prepared brain tissues, mice were not perfused, and the brains were directly blocked and shortly fixed in 4% paraformaldehyde (wt/vol) for 30 min and then maintained in 30% sucrose (wt/vol) before sectioning on cryostat. The brains were sectioned at 15 μm. The sectioned tissues were stored at −20 °C before staining and discarded after 1 month. For paraffin embedded brain tissue preparations, mice were transcardially perfused with a fixative of 2% paraformaldehyde and 0.2% picric acid (wt/vol) in 0.1 M sodium phosphate, pH 7.2, and the brains were postfixed in 4% paraformaldehyde overnight at 4 °C. The brains were preserved in 70% ethanol for 2 h and blocked and then processed by the Vanderbilt Pathology core with the standard procedure in the core. Paraffin embedded tissues were sectioned at 5 μm using a microtome and stored at 22–26 °C. The sectioned brain slices were mounted on SuperFrost Plus slides for histochemical and immunohistochemical studies. The brain slices were routinely deparaffinized, rehydrated and antigen retrieved for 10 min at 95 °C. The slices were then blocked for endogenous peroxidase activity with 3% H2O2 (wt/vol) solution for 10 min, permeabilized with 0.4% Triton X-100 (vol/vol) for 10 min and blocked with 0.2%/0.2% BSA/Triton X-100 for 1 h followed by immunoreaction with specifically targeted antibodies overnight. The slices were then gently washed with 0.1% BSA/PBA (wt/vol) three times before incubating with secondary antibodies. The slices were then processed with gradient alcohol (75%, 95% and 100%) and rinsed with the hydrophobic clearing agent xylene for 2 min twice and DAB reaction before being sealed with coverslips or directly sealed with ProLong Gold antifade mounting medium (Molecular Probes) for slices with fluorophore conjugated antibodies. The nuclei were either counterstained with hematoxylin for bright microscopy or with Topro-3 (1:500) for fluorescence microscopy.

Immunohistochemistry related quantifications. For all γ2 subunit and caspase 3 protein intensity measurements, raw values of the protein intensity in the somatic region were measured in ImageJ. A total of 10–12 randomly chosen non-overlapping fields from three consecutive sections per mouse were
measured each time. All the neurons were included in each field. The mean value was taken as n = 1 for each mouse. If the staining was with fluorophore-conjugated secondary antibodies, the nuclear region of the same cells was used as background because the boundary of the nucleus was not as clear as the plasma membrane. The protein intensity value of the somatic region for each cell was equal to the value of the somatic region subtracting the background value. In Figure 6c, the non-somatic fluorescence density value was the direct measurements of the neurite region. The cell counting for caspase 3-, calbindin D-28K– and TUNEL-positive cells as well as the NeuN-positive cells colabeled with cortical layers V or VI mark- ers Er81 and Trb1 were quantified based on our previous study47. The mean positive staining was determined by counting 10–12 non-overlapping fields in specific layers in brain sections or in randomly chosen fields in cultures under 63× objective. The 10–12 non-overlapping fields were from three sections in each mouse or three dishes for each genotype in cultures for each experiment. The counts of three sections or three dishes were averaged and taken as n = 1. For the quantifications of the protein intensity in the somatic region in the immunohis- tochemistry, ImageJ was used.

Digital histology and unbiased cell counting. For the brain sections with good representation of somatosensory cortex zone 1 and 2 (S1–2), we used the interaural 2.22 mm, Bregma 1.58 mm reference point based on the mouse brain atlas and cut 6–8 rostral sections at intervals of 5 µm from each mouse brain for the assay in cortex, hippocampus and thalamus. We prepared the cerebellar sections separately. The mouse brains were cut sagittally across the midline and 6–8 consecutive sections at intervals of 5 µm were prepared parasagittally starting from the midline in the right hemisphere. The images were acquired automatically by computer, and the image processing parameters were determined by the central core personnel who were blind to mouse genotype. All the images were scanned at 20x and viewed with 7x. The total number of 5 × 105 µm2 in somatosensory cortex were quantified manually by a person blind to genotype or by MetaMorph software. The cell counts of the 6–8 consecutive sections were averaged and taken as one of five pairs of mice.

Subcellular fractionation and isolation of synaptosomes. The procedures of subcellular fractionation were modified from a previous study48. Briefly, after quickly removing the brains, regions of interest were dissected and homogenized in pre-chilled homogenization HEPES-buffered sucrose buffer (HBS containing 0.32 M sucrose, 10 mM HEPES, pH 7.4, 2 mM EDTA) with a ratio of 10–12 ml buffer: 1 g of brain tissue (each mouse brain is around 0.4 g) with 10–15 strokes. The homogenates were then spun down at 1,000 g for 10 min to remove the cell debris and nuclei. The pellet was taken as P1. The supernatant was taken as S1, and S1 was centrifuged at 13,800 g for 20 min. The S1 pellet was taken as P2, and the supernatant was taken as S2. S2 was the combination of cytosol and light membranes, while P2 was crude synaptosomal fraction. P2 was resuspended with HBS buffer at 1:10 ratio and centrifuged at 13,800 g for 15 min (for wash). The washed pellet was then mixed with 9× vol ice cold H2O-protease inhibitors. After rapidly adjusting to 4 mM HEPES by using 1 M HEPES (pH 7.4), the P2 suspension was then rotated for 30 min at 4 °C, followed by spinning at 25,000 g for 20 min. The pellet was taken as P3 and resuspended by adding 1.5 ml HBS buffer followed by discontinuous gradient sucrose ultracentrifuge. An equal volume (2.2 ml) of sucrose with different concentrations (1.4 M, 1.2 M, 1.0 M to 0.8 M) was added from bottom to top in the tube. The sample (1.5 ml) of P3 was added to the top of the sucrose layers. The sucrose tube containing the sample of P3 was then centrifuged at 150,000 g for 1 h, and then each layer of interest was carefully collected and stored at –80 °C. The synaptosome layer (spm) was at 1.0/1.2 M sucrose interface.

Live brain slice biotinylation. The protocol was based on a previous study49. Briefly, the brain slices were incubated for 45 min at 4 °C in artificial cerebrospinal fluid (aCSF) that contained 1 mg/ml of the membrane-impermeable, biotinyl- ation reagent, sulfo-NHS-biotin (Thermo Scientific). After biotinylation, the slices were washed with 0.1 M glycine in aCSF. The cortices were dissected based on the region of interest and sonicated in radioimmunoprecipitation assay (RIPA) solution (20 mM Tris, pH 7.4, 1% Triton X-100, 250 mM NaCl) that also contained protease inhibitor mixture (1:100, Sigma-Aldrich), 0.5% deoxycholate (wt/vol) and 0.1% SDS (wt/vol).

Brain slice preparation and recording. Recordings of GABAergic mIPSCs were obtained from layer V1 somatosensory cortex neurons in brain slices as previously described50. Coronal brain slice sections (300 µm) were made using a vibratome in ice-cooled solution containing (in mM) 214 sucrose, 2.5 KCl, 1.25 NaH2PO4, 0.5 CaCl2, 10 MgSO4, 24 NaHCO3, and 11 D-glucose, pH 7.4 buffered with 95%O2/5%CO2 at 4 °C. Slices were then incubated in oxygen- ated aCSF at 36 °C for 30 min. After this, slices were kept for at least 1 h before recording on a Nikon Eclipse FN-1 IR-DIC microscope at 22–26 °C. Pipette internal solution contained (in mM): 135 CsCl, 10 EGTA, 10 HEPES, 5 ATP-Mg, and QX-314 (5 mM) (pH 7.25, 290-295 mOsm), and recording micropipette resistances were 2–4 MΩ. Whole-cell patch clamp recordings were made at 22–26 °C from layer VI pyramidial neurons in S1/II somatosensory cortex visualized with an upright Nikon eclipse FN-1 IR-DIC microscope. Neurons in the layer above the white matter with a typical apical dendrite and large soma (smaller than the layer V neuronal somata) were chosen for recordings. Data were collected using a MultiClamp 700B amplifier with compensation for series resistance (70%) and cell capacitance. Data were filtered at 2 kHz and digitized at 20 kHz using a Digidata 1440A AD converter.

Synchronized EEG recordings and analysis. Synchronized video EEGs were recorded from 8-week-old to 4-month-old C57BL6J mice 1 week after electrode implantation and recorded with synchronized EEG monitoring system from Pinnacle Technology based on previous study49. Briefly, mice were anesthetized with 1–3% isoflurane (vol/vol) and four epidural electrodes (stainless-steel screws affixed to one head mount) were placed on the brain surface and secured in place with dental cement and surgical stitches. EMG leads were inserted into the trapezius muscle. Mice were allowed to recover from the EEG head mount implantation surgery for one week before EEG recording. Video-EEG monitoring lasted for 24–48 h, and mice were freely moving during EEG recordings. Mouse behaviors such as behavioral arrest, myoclonic jerks or generalized tonic-clonic seizures during the EEG recordings were identified to determine if mice exhibit behavioral seizures. At least 24 h of baseline EEG recordings were obtained and analyzed for each mouse.

Open field test. Open field test was performed based on the standard protocol in Vanderbilt Neurobehavior core. Briefly, each individual mouse was placed into an open field activity chamber (Med Associates, 27 × 27 × 20.3 cm) that contained within light- and air-controlled environmental chambers for 60 min. Location and movement were detected by the interruption of infrared beams by the body of the mouse (16 photocells in each horizontal direction, as well as 16 photocells elevated 4 cm to measure rearing) and were measured by the Med Associates Activity Monitoring program. Total distance traveled and time resting in center (50% of area) and peripheral zone were measured. The open field arena was cleaned with 70% ethanol and wiped with paper towels between each trial.

Elevated zero maze test. The elevated zero maze is a modification of the elevated plus maze used for assessing anxiety-related behaviors. The elevated circular platform (40 cm off the ground, 50 cm in diameter) had two enclosed arenas opposite each other (5 cm wide with 15 cm high walls) and two open arenas (5 cm wide). Briefly, each mouse was lowered by its tail into the open arena of the maze and allowed to explore the maze for 300 s. The whole circular platform was cleaned with 70% ethanol and wiped with paper towels between each animal. Activity of the mouse was monitored via an overhead camera connected to a computer in a separate room using video acquisition and ANY-maze analysis software (Stoelting). Data analyzed included time spent in the open versus closed arms, the number of entry in the open arms.

Protein structure prediction and docking. We used MUFOLD50 to predict the protein structures. The docking was performed by symmetric docking of SymmDock between two γ2 subunits and by general docking of PatchDock between the γ2 and α1 subunits51. Chimera (UCSF Chimera, a visualization system for exploratory research and analysis52) was used to display the protein structures.
**Statistical analysis.** IDVs were quantified by using the Quantity One or Odyssey fluorescence imaging system (Li-Cor). The fluorescence intensity values were quantified by using ImageJ. Data were expressed as mean ± s.e.m. values and analyzed with Graphpad Prism 5.0 software. Statistical significance of immunoblot and cell imaging data was determined by two-way ANOVA with Bonferroni post-tests, a Student's unpaired t test or, if appropriate, single-value t test. For the comparison of GABAergic mIPSC electrophysiology data, a Kolmogorov-Smirnov test was used to determine the cumulative probabilities, the distribution of wild-type and the mutant mIPSC peak amplitudes. The behavioral data was analyzed by a distribution-free Mann-Whitney U test as well as Student's t test. No statistical methods were used to pre determine sample sizes, but our sample sizes are similar to those reported in previous publications\textsuperscript{10,19,20}. Data distribution was assumed to be normal, but this was not formally tested. All analyses used an alpha level of 0.05 to determine statistical significance.

A Supplementary Methods Checklist is available.

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