Pathomechanisms for *GABRG2* and *GABRB3* Mutations in DEEs

Subjects: Neurosciences

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$GABA_A$ receptor genes (*GABR*) are a group of genes associated with DEE, although previous studies have been focused on relatively mild epileptic syndromes, such as childhood absence epilepsy (CAE), febrile seizures (FS), and generalized tonic-clonic seizures with febrile seizure plus (GEFS+). Mutations in $GABA_A$ receptor subunit genes (*GABRs*) are a major etiology for developmental and epileptic encephalopathies (DEEs).

Keywords: GABRB3 ; GABRB2 ; DEEs

1. Pathomechanisms for *GABRG2* Mutations in Developmental and Epileptic Encephalopathies

The most common phenotypes associated with *GABRG2* mutations are febrile seizures, generalized tonic-clonic seizures with febrile plus (GEFS+), and DS, with febrile seizures being the core phenotype (**Figure 1**A). Extensive work from previous studies has shown that *GABRG2* mutations are associated with a wide spectrum of seizure disorders (**Figure 1**B). There has been much investigation into the impact of *GABRG2* mutations in vitro and in vivo using knock-in mouse models, which would allow researchers to infer the pathophysiology of this mutation in the patient.

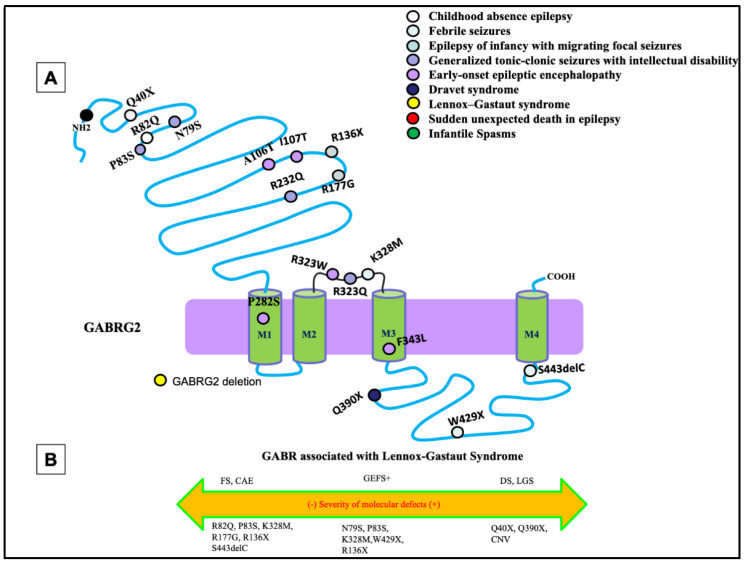

Figure 1. Epilepsy phenotypes associated with *GABRG2* mutations. (**A**) Two-dimensional protein topology of the γ_2 subunit showing locations of mutations in human *GABRG2* associated with various epilepsy syndromes and neurodevelopmental disorders. Each mutation is color coded to indicate associated diagnosis, and each dot represents the relative location on the γ_2 subunit protein peptide. (**B**) Diagram dictating the severity of each mutation on a continuum of epilepsy phenotypes. The left side of the diagram infers a less severe epileptic phenotype, the middle dictates a moderate, and the right side of the diagram infers a more severe epileptic phenotype.

Mutations of *GABRG2* are frequently associated with developmental and epileptic disorders through both missense and nonsense mutations. Nonsense mutations in *GABRG2* have been identified and are associated with FS, GEFS+, or DEEs. A *GABRG2* (*R43Q*) mutation had reduced cell surface expression of the subunit and reduced cortical inhibition in knock-in mice [1][2]. However, in some cases those mutant receptors, which traffic to the cell surface, have normal function [3]. It was also identified that a subset of mutations, especially the nonsense mutations *GABRG2* (Q351X), cannot traffic to the cell surface and synapse due to severe misfolding [4][5]. The mutant protein was retained in the endoplasmic reticulum and reduced trafficking of wildtype partnering subunits, implicating its dominant-negative suppression causing GEFS+.

The mutant *GABRG2* (Q351X) has slow degradation and can impose a dominant-negative effect on the wildtype *GABRG2* allele and the partnering subunits [4]. These findings have been validated in vivo with *Gabrg2*$^{+/Q390X}$ mice, showing that the mutation *GABRG2* (Q390X) is proven to cause chronic subunit accumulation and neurodegeneration [5]. In older mice, the mutated subunit was shown to form protein aggregates due to chronic accumulation of the mutant protein in the endoplasmic reticulum [5]. Mice expressing the *GABRG2* (Q390X) mutation showed a severe epileptic phenotype, which included spontaneous generalized tonic-clonic seizures in a seizure-resistant C57BL/6J mouse background. The phenotype is much more severe than the *Gabrg2*$^{+/-}$ heterozygous knockout mice without generation of the mutant protein [6].

As mentioned above, the γ_2 subunit plays an essential role in GABAergic neurotransmission and the overall homeostasis of the central nervous system, while impairment in the subunit results in seizures and neurodevelopmental abnormalities. Some mutants of this protein display a dominant-negative effect that suppresses expression of both wildtype subunit and its binding partners in the hetero-pentamer. Of note, the proband displays an uncharacteristic phenotype for a mutation of this subunit, suggesting further investigation into the interplay of associated proteins with the receptor and how their function is affected by the mutation. The $GABA_A$ receptor exerts channel function as a pentamer, and the γ_2 subunits oligomerize and colocalize with partnering subunits. There is the possibility that the deficiency due to mutant γ_2 protein can result in suppression of proper function of the β_3 subunit whose perturbation is often attributed to the LGS phenotype.

2. Pathomechanisms for *GABRB3* Mutations in Developmental and Epileptic Encephalopathies

Like the phenotypic heterogeneity associated with mutations in *GABRG2*, mutations in *GABRB3* have also been associated with a spectrum of disease phenotypes, including ASD, IS, and DS [7][8] (**Figure 2**A,B). Among all *GABR*s, only mutations in *GABRB3* have been previously reported to be associated with LGS [9]. In addition to LGS, the β_3 subunit encoded by *GABRB3* has also been frequently associated with CAE [10][11]. In addition to the defect in the mutant β_3 subunit, a mutant β_3 subunit can also compromise the assembly and trafficking of partnering subunits α_1 or γ_2. This suggests there may exist overlapping phenotypes at the clinical level among mutations in the partnering subunits of the receptor through a dominant-negative effect of one, suppressing the maturation and trafficking of the others, as previously reported [6].

Figure 2. Epilepsy phenotype associated *GABRB3* mutations. (**A**) Two-dimensional protein topology of the β_3 subunit showing relative locations of mutations in human *GABRB3* associated with various epilepsy syndromes and neurodevelopmental disorders. Each mutation is color coded to indicate associated diagnosis, and each dot represents the β_3 subunit protein peptide. (**B**) Diagram dictating the severity of each mutation on a continuum of epilepsy phenotypes. The left side of the diagram infers a less severe epileptic phenotype, the middle dictates a moderate, and the right side of the diagram infers a more severe epileptic phenotype.

Mutations in *GABRB3* have been frequently related to DEEs (**Figure 2**). A previous study evaluated the mutations identified by the Epilepsy Phenome/Genome Project (D120N, E180G, and Y302C) [7]. Follow-up functional assays

identified the alterations of GABA-activated channel function, including reduced GABA-evoked current amplitudes, slowed activation, and accelerated deactivation [12]. This was inferred to be due to a reduction in the GABA potency, representing the concentration of GABA, and efficacy, representing the potential of GABA to bind, caused by the mutations. Specifically, the D120N mutation reduced GABA potency, whereas the E180G and Y302C mutations reduced GABA efficacy [12]. This implicates that different mutations can have separate effects on the inhibitory neurotransmission mechanisms. It is important to note that each mutation was in either loop A or loop B of the GABA-binding pocket or the M2-M3 loop, which is involved in the ligand-binding channel, gating-coupling mechanism. The specific pathomechanism could be the likely cause of the mutations disrupting coupling of GABA binding to channel gating.

The differential pathomechanisms in *GABRB3* mutations associated with epilepsy with variable severities have been identified [13]. There was a thorough comparison of the *GABRB3* (N328D) mutation associated with LGS with *GABRB3* (E357K), a mutation associated with a less severe phenotype: juvenile absence epilepsy [13]. In the patient carrying the N328D mutation, there was presence of generalized tonic-clonic seizures and myoclonic seizures. The initial EEG examination showed ictal 2–2.5 Hz generalized polyspike-wave discharges during myoclonic seizures, interictal spike, and slow wave complexes, and irregular fast rhythms with slow waves were recorded during wakefulness [13]. It was identified that the LGS-associated *GABRB3* (N328D) mutation caused more reduced cell-surface expression and synaptic presentation of $\alpha_1\beta_3\gamma_2$ than *GABRB3(E357K)* mutation associated with the juvenile absence epilepsy. However, both mutations caused trafficking defects due to endoplasmic reticulum retention of the mutant protein. Through use of a high-throughput flow cytometry assay, the surface expression of β_3 and γ_2 subunits for several *GABRB3* mutations was evaluated. There was a significant reduction in the expression of the β_3 and γ_2 subunits at the cell surface for both mutations [13]. This implies that a mutation of this subunit not only prevents proper trafficking of the individual subunit itself but for the partnering wildtype subunits such as γ_2 and α_1 subunit expression, suggesting loss of whole receptor function instead of subunit alone. By comparing mutations in *GABRB3* associated with different phenotypes or different mutations with the same phenotype such as LGS, it is likely that different mutations can have separate effects on the inhibitory neurotransmission mechanisms.

The impact of the *GABRB3* mutation associated with LGS has also been investigated in mutant, knock-in *Gabrb3*$^{+/D120N}$ mice [9]. Like seizures observed in patients carrying the mutation, multiple types of spontaneous seizures including absence, myoclonic, tonic, and generalized tonic-clonic seizures were observed in mice of approximately four months of age. More specifically, the mutant mice showed a seizure frequency of 445 absence, 99 myoclonic, and 4 tonic seizures [9]. As there is a neuropsychiatric comorbidity in LGS, the disease-relevant behavioral abnormalities commonly observed in LGS such as ID and ASD have been evaluated with a battery of behavioral tests conducted in *Gabrb3*$^{+/D120N}$ mice. The *Gabrb3*$^{+/D120N}$ mice displayed impaired sociability and cognition evaluated with the three-chamber socialization test. In the Barnes maze test, the *Gabrb3*$^{+/D120N}$ mice showed a longer latency to find the target hole and increased number of errors in the learning trials. In the memory trial, there was also a significant increase in the number of errors to find the target hole, which both suggests learning and memory deficits in the mice [9].

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