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Case Report

A case of *GABRA5*-related developmental and epileptic encephalopathy with response to a combination of antiepileptic drugs and a GABAering agent

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Abstract

Background: $GABA_A$ receptors are ligand-gated chloride channels that regulate inhibitory neurotransmission in the central nervous system. Recently, monoallelic *de novo* mutations in *GABRA5* resulting in altered inhibitory synapses were found in three patients with developmental and epileptic encephalopathy.

Patient description:

We report on a four-year and six-month-old girl with epilepsy and global developmental delay. Serial head growth measurement revealed postnatal onset microcephaly.

Results: Magnetic resonance imaging (MRI) of the brain was normal at the age of eight months and subsequently showed a decrease in white matter volume and thin corpus callosum at the age of 3 years. Using whole-genome sequencing, we identified the fourth patient harboring a *de novo* missense mutation in *GABRA5*. Interestingly, the c.880G > C (p.V294F) affects the same position found in two of the three previously reported patients.

Conclusion: This study suggests that the nucleotide c.880G is a mutation hotspot. Our patient also demonstrated significant seizure reduction after benzodiazepine. To our knowledge, this is the first case describing the favorable outcome of a GABAergic agent in seizure control for *GABRA5*-related developmental and epileptic encephalopathy.

Keywords: Developmental delay; Epileptic encephalopathy; $GABA_A$ receptor; *GABRA5*

1. Introduction

GABA (gamma-aminobutyric acid) serves as the primary inhibitory neurotransmitter of the central nervous system. $GABA_A$ (the gamma-aminobutyric acid receptor type A) receptors are ligand-gated anion channels that are activated by GABA to mediate inhibition of neurotransmission [1]. Mutations in the genes encoding

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the GABA receptor subunits have been associated with various types of epilepsy, including juvenile myoclonic epilepsy, childhood absence epilepsy, febrile seizures, and early-onset epileptic encephalopathy [2,3]. *De novo* pathogenic mutations in *GABRA1, GABRA2 GABRB2, GABRB3, and GABRG2* were associated with infantile-onset epilepsy, including Ohtahara syndrome, infantile spasms, Dravet syndrome, and Lennox–Gastaut syndrome [1]. Contribution of missense variants in *GABRA5* to genetic generalized epilepsy, infantile-onset epilepsy and developmental delay has been recently established [4–6]. There have been three patients with early-onset epilepsy and profound developmental delay harboring *GABRA5* mutations reported in the literature. Here, we identified a Thai patient with developmental and epileptic encephalopathy with a previously reported *de novo* missense variant c.880G > T (p.V294F) in *GABRA5*. This finding suggests that the nucleotide c.880G is a mutation hotspot. Our patient also showed significant seizure reduction after benzodiazepine. To our knowledge, this is the first case describing the favorable outcome of a GABAergic agent in seizure control for *GABRA5*-related developmental and epileptic encephalopathy.

2. Case presentation

The patient is a four-year and six-month-old girl. She is the only child of unrelated parents with no family history of epilepsy. The father and the mother were 33 and 38 years old, respectively, when the patient was born. She was delivered at term via caesarian section. The birth weight was 3,100 g, APGAR scores were 9 and 10 at 1 min and 5 min, respectively. Head circumference at birth was 35 cm. A 1-cm oval-shaped alopecic area on the occipital scalp was noted. The rest of the physical examination was unremarkable at birth. At age two months, she developed multiple episodes of tonic seizures and facial twitching. Global developmental delay was noted since the onset of seizures. Serial head growth measurement revealed postnatal onset microcephaly (Fig. 1A). Magnetic resonance imaging (MRI) of the brain was normal at age eight months and subsequently showed a decrease in white matter volume and thin corpus callosum at the age of 3 years (Fig. 1B). An electroencephalogram (EEG) performed at age four months revealed no epileptiform discharge. Video-EEG at age eight months showed multiregional sharp waves at bilateral parieto-temporal regions. At age three years, very frequent epileptiform discharges at the fronto-temporo-occipital regions were noted (Fig. 1C). Ictal EEG showed diffused low amplitude followed by bitemporal sharp discharges, and fast activities (Fig. 1D) correlated with sudden jerking, head turning, and tonic posturing of the left arm, evolving to generalized tonic-clonic seizures. Five episodes of electroclinical

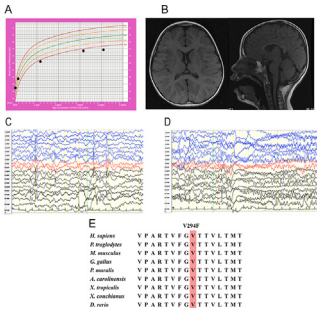

Fig. 1. Clinical, neurophysiological and imaging features. Head size measurements showed postnatal onset microcephaly (A). Brain MRI showed decreased white matter volume and hypoplasia of corpus callosum on sagittal and axial views at age 3 years (B). EEG tracing from the subject at age 3 years showing multifocal epileptiform discharges from bilateral parieto-temporal area (sensitivity 20 mV) (C) Ictal EEG showed diffused low amplitude followed by bitemporal sharp discharges and fast activities (D). Evolutionary conservation of amino acid across different species. The valine residue at codon 294 is indicated in red bar (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

seizures were captured during 4-hour-monitoring. Seizures were refractory to phenobarbital, topiramate, and levetiracetam in combination. The first drug prescribed at the age of four months was phenobarbital at a dose of 5 mg/kg/day. At the age of six months, seizures were still frequent. In treating refractory epilepsy, we aim to choose drugs that target different seizure mechanisms for synergistic effects. Topiramate which acts on various mechanisms including antagonism of α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA)/kainite receptors, augmentation of GABA activity, and blocking of voltage-gated sodium channels was added. Topiramate was titrated to 10 mg/kg/day. The seizures were still refractory. The third anti-epileptic drug added at the age of ten months was levetiracetam which binds to the synaptic vesicle protein SV2A and decreases the neurotransmitter release. Levetiracetam was titrated to 75 mg/kg/dose. Despite the combination of three anti-epileptic drugs at optimum doses and good compliance, frequent seizures up to 10–20 episodes per day were still observed and confirmed by EEG monitoring. Clobazam acts on the γ-aminobutyric acid ($GABA_A$) by increasing the frequency of GABA-mediated chloride channel openings, a different manner of phenobarbital which acts on prolonging the opening of the chloride channel. The efficacy and safety of clobazam as an add-on therapy in pediatric refractory epilepsy have been previously explored; it is therefore chosen as the fourth medication [7]. At two years and nine months old, clobazam was titrated up to 1.5 mg/kg/dose and resulted in seizure reduction to 2–3 episodes per day. The patient is non-verbal and has not achieved neck muscle control at the age of four years. Social smiles are present.

Informed consent for genetic testing was obtained before blood samples from the patient and the parents were collected. This study was approved by the institutional review board (IRB No. 054/60) of the Faculty of Medicine, Chulalongkorn University, Thailand.

3. Molecular genetic analysis

Trio whole-genome sequencing of the patient and her parents was done. We followed a variant prioritization using Exomiser (https://github.com/exomiser/Exomiser) to identify causative variants. HPO terms (HP:0001263; global developmental delay, HP:0200134; epileptic encephalopathy and HP:0001250; seizures) were used. The *de novo* variants were first targeted. A heterozygous *de novo* c.880G > C (p.V294F) variant in *GABRA5* was identified. The variant was absent in population controls (gnomAD; https://gnomad.broadinstitute.org/) and the in-house database comprising 2,166 unrelated Thai exomes. The prediction tools suggest the variant to be disease-causing (Polyphen2: 0.998, Mutation Taster: 1.000). The variant occurred *de novo* (PS2) and there has been a well-established *in vivo* functional study to support the damaging effect (PS3) [5]. The variant is therefore classified as "pathogenic" according to the ACMG criteria [8].

4. Discussion

Rare variants in *GABRA5* have previously been identified in patients with generalized epilepsy and autistic spectrum disorders. Recently, the c.880G > C (p.V294L), c880G > T (p.V294F) and c.1238C > T (p.S413F) in *GABRA5* were found in three patients with infantile-onset epilepsy and developmental delay [4,5]. We here describe the fourth patient. She is a Thai patient with severe developmental delay and epilepsy starting at age two months carrying a heterozygous *de novo* missense variant c.880G > T (p. V294F) in *GABRA5*. This finding affirms *GABRA5* as a gene causing severe epilepsy phenotype. The phenotype of our patient resembles the three previously reported cases. Intractable seizures developed during infancy and severe neurocognitive disability ensued. Microcephaly with cortical atrophy and hypomyelination has been reported in previous cases. The comparative clinical data are shown in Table 1.

Many lines of evidence suggest that the c.880G > T (p.V294F) in *GABRA5* is pathogenic. It was absent in population controls (http://exac.broadinstitute.org/gnomAD; https://gnomad.broadinstitute.org/) and the in-house database comprising 2,166 unrelated Thai exomes. The c.880G > T resulted in the substitution of phenylalanine for valine at codon 294 (p.V294F) within the M2 transmembrane domain forming the ion channel pore of the receptor. The affected valine is conserved across different species (Fig. 1E) [5]. The effect of the variant on channel function has been previously examined. Hernandez et al. demonstrated that the p.V294F mutation in *GABRA5* resulted in the decrease in numbers of mutant $GABA_A$ receptors at the postsynaptic sites and reduced the amplitude of $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents in the hippocampal neurons. As $GABA_A$ clustering and inhibitory function play a vital role in neuronal network development, the mutant receptors lead to brain hyperexcitability [5].

Theoretically, $GABA_A$ receptor modulators, such as benzodiazepines and barbiturates, may have less benefit in patients with *GABRA5* mutations because these drugs need to bind to $GABA_A$ receptors in order to enhance its inhibitory function. In patients with mutant and decrease GABA receptors, targeting non-GABAergic pathways is a more rational choice of treatment. Butler et al. reported a patient harboring p.V294L variant in *GABRA5* who achieved seizure freedom on a combination of zonisamide, levetiracetam, and oxcarbazepine. In contrast, our patient showed seizures reduction from 10 to 20 episodes per day to 2–3 episodes per day after adding clobazam to the combination of phenobarbital, levetiracetam, and topiramate. Although it is unclear whether clobazam, the combination therapy, or the natural course of the disease contributes to seizure reduction in this patient, our findings suggest that mutations in *GABRA5* do not entirely preclude $GABA_A$ receptor modulators prescription. $GABA_A$ receptor modulators may still have a positive effect on seizure reduction despite the mutant and the decrease amount of GABA receptors.

Parent-child exome sequencing studies in sporadic developmental and epileptic encephalopathy (DEE) patients have shown that *de novo* mutations (DNMs) are an important etiology of DEE. *De novo* missense variants contribute a large proportion of disease-causing variants and these variants are frequently recurrent [9]. Two previously reported cases harbor a heterozygous *de novo* c.880G > T(p.V294F) and a c.880G > A (p.V294L) variant. We identified another patient harboring a *de novo* c.880G > T (p.V294F) mutation in *GABRA5*. This suggests that the nucleotide c.880G is a mutation hotspot.

The mutation hotspot might also reflect the structural and functional features of the respective DNA sequences. The valine to phenylalanine at the amino acid residue 294 is located within the M2 transmembrane domain forming the ion channel pore of the receptor. The fact that this *de novo* variant has been identified in three individuals with a similar phenotype also suggests that it confers a specific property to the protein.

Table 1
Characteristics of developmental and epileptic encephalopathy patients with variants in *GABRA5*.

Subject	**This patient**	**Hernandez et al. 2019**		**Butler K.M. et al. 2018**
Gender	Female	Male	Male	Male
Seizure onset	2 months	4 months	3 months	4 months
Analysis	WGS	Gene panel	Gene panel	WGS
Genomic position	Chr15:27188364	Chr15:27188364		Chr15:27188364
Mutation	c.880G > T	c.880G > T	c.1238C > T	c.880G > A
Substitution	p. Val294Phe	p. Val294Phe	P. Ser413Phe	p. Val294Leu
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*
Severe delay/ Intellectual disability	Severe delay	Severe delay	Severe delay	Delayed milestones, non-verbal autistic features
Seizures	Facial twitching, arm jerking, oral automatism, generalized tonic, generalized tonic clonic	Focal seizures with generalization, febrile seizures, and statusepilepticus	Focal and tonic seizures. epileptic spasms	Myoclonic, tonic, tonic-clonic seizures, oral automatism, migratingpartial seizures
Anti-epileptic drugs	PB + LEV + TPM + clobazam (>50% seizure reduction)	Data not shown	Data not shown	PB, TPM (unresponsive), LEV + ZNS+OXC (seizure-free)
EEG	Multifocal epileptiform discharges predominantly at the fronto-temporal area	Generalized slow waves in background activity, multifocal sharp waves	hypsarrhythmia	Epileptiform discharges predominantly at the temporal and posterior parts of the brain
MRI	A decrease in white matter volume and thin corpus callosum.	Frontotemporal atrophy and thin corpus callosum	Cortical atrophy and thin corpus callosum	Hypomyelination
Other findings	Postnatal onset microcephaly, hypotonia	NA	NA	Postnatal onset microcephaly

Genomic positions are relative to the GRCh37/hg19 human genome assembly. Abbreviations and symbols are as follows: EEG = electroencephalography; LEV = levetiracetam; MRI = magnetic resonance imaging; OXC = oxcarbazepine; PB = phenobarbital; TPM = topiramate; WGS = whole-genome sequencing; ZNS = zonisamide; NA = not available
+ Indicate combination of anti-epileptic drugs.

5. Conclusion

We identified the fourth patient with severe epilepsy phenotype and a *GABRA5* mutation, suggesting that the nucleotide c.880G is a mutation hotspot. The patient's seizures were responsive to clobazam. Our findings suggest that mutations in *GABRA5* do not entirely preclude $GABA_A$ receptor modulators prescription.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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