Original Article

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Identification of novel and *de novo GABRB1* mutation in Chinese patient with developmental and epileptic encephalopathy 45

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SUMMARY Developmental and epileptic encephalopathy 45 (DEE45) is an autosomal dominant disease caused by variation in the gamma-aminobutyric acid type A receptor subunit beta 1 (*GABRB1*) gene. Affected individuals have severely impaired intellectual development, hypotonia, and other persistent neurological deficits. However, DEE45 is rare; only four infants with DEE45 have been reported worldwide and no case has been reported in China. Confirming a diagnosis of DEE45 is of great significance for guiding further treatment, assessing patient prognosis, and genetic counseling. The clinical characteristics of DEE45 and the medical history of DEE45 patients requires supplementation and clarification. Here, we present the clinical and genetic findings of a 7-year-old girl with DEE45 carrying a novel *de novo GABRB1* mutation (c.858_859delinsTT, p.286_287delinsIleSer) identified by whole exome sequencing (WES). The mutation is phylogenetic conserved in the second helix of the β1-subunit's transmembrane region. Western blot and RT-qPCR both indicated significant increase in the expression levels of GABRB1 mutant when compared with wild. The proband has epileptic encephalopathy and experienced refractory epilepsy onset at age 2 months and showed developmental delay at age 8 months. Electroencephalography (EEG) displayed hypsarrhythmia. Magnetic resonance imaging (MRI) showed no significant abnormalities in the internal structure of the patient's brain, which is displayed in two previously reported cases. The patient's symptoms of hypotonia, ataxia, profound mental retardation, and dysmetria became evident with development. In summary, we report the genetic and clinical characteristics of the first Chinese patient with DEE45 and explores the relationship between mutation and clinical symptoms.

Keywords DEE45, *GABRB1*, *de novo* mutation, developmental delay, epilepsy of infancy, electroencephalography

1. Introduction

Epileptic encephalopathies include a large group of severe epileptic syndromes with a broad phenotype spectrum. At present, the incidence of epileptic convulsive state, non-convulsive state epilepsy, and sudden unexplained death in epilepsy for these diseases is unknown (*1*). DEE45 is a neurological disorder characterized by significant total developmental retardation in infancy or early childhood and seizures in the first 12 months of life, with frequent mental retardation, functional impairment, and other persistent neurological deficits (*2*). It is caused by a heterozygous variation in the *GABRB1* gene, which encodes a subunit of the gamma-aminobutyric acid type A receptor ($GABA_A$) (*3*). $GABA_A$ is a ligand-gated chloride ion channel that mediates GABAergic inhibition in the central nervous system and is involved in controlling neuronal excitability in the brain and spinal cord (*4,5*). The binding of GABA to a $GABA_A$ receptor can quickly open its CI channel and mediate rapid inhibitory synaptic transmission in the central nervous system (*6*). Changes in the quantity, distribution, and dynamic properties of $GABA_A$ receptors at the synapse are key mechanisms for regulating the intensity of inhibitory synaptic transmission and neural circuit information processing (*7*).

To date, four variations in *GABRB1* have been identified in patients with DEE45, with no patients reported in China. Here, we report the clinical and

genetic findings of a fifth patient with *GABRB1*-related DEE45. This study expands the *GABRB1* variation spectrum and clinical symptoms of this rare disease and contributes to understanding the phenotype genotype relationship of the disease.

2. Subjects and Methods

2.1. Study subjects

This study was approved by the ethics committee of Shandong First Medical University & Shandong Academy of Medical Sciences. We certify that the study was performed in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient's family. Peripheral blood samples were collected from the patient and her parents. The patient's medical records, clinical examination and treatment were reviewed.

2.2. Genetic analysis by WES and Sanger sequencing

Genomic DNA was extracted from blood samples using the TIANamp Blood DNA Kit (TIANGEN Biotech Co., Beijing, China). WES of the proband was performed on the Illumina Novaseq 6000 platform (Illumina Inc., San Diego, CA, USA). After sequencing, base-call file conversion and demultiplexing were conducted using bcl2fastq software (Illumina). The resulting fastq data were analyzed by in-house quality control software to remove low quality reads, and were then aligned to the reference human genome (hs37d5) using the Burrows-Wheeler Aligner (BWA) (*8*), and duplicate reads were marked using Sambamba tools (*9*). Single nucleotide variants (SNVs) and indels were called with GATK (*10*). Copy number variant (CNV) data were detected using the SVD-ZRPKM algorithm, CoNIFER (version 0.2.2) (*11*). Mutation Taster was used for predication of mutation effect (*12*).

After filtering for rare variants, the pathogenicity of identified disease-attributable gene variants was evaluated using the updated guidelines for the interpretation of molecular sequencing from the American College of Medical Genetics and Genomics. The candidate variants were validated in the proband and her parents by Sanger sequencing, which was conducted by Beijing Liuhe BGI.

2.3. Phylogenetic analyses and Three-dimensional (3D) structural model predication

Amino acid sequences among human α1-6, β1-3, and γ1-3 GABAA receptor subunits and amino acid sequences of GABRB1 from ten different species were drawn from National Center for Biotechnology Information website and then aligned using software of Molecular Evolutionary Genetics Analysis (MEGA) and GeneDoc with default setting (*13*). The phylogenetic tree was drawn using MEGA software. Three-dimensional structural model of variant was predicted by AlphaFold Protein Structure Database (*14*).

2.4. *In vitro* expression of *GABRB1*

To explore the effect of the identified *GABRB1* variation (858_859delinsTT) on the expression of *GABRB1*, human embryonic kidney 293 (HEK293) cells were transfected with plasmids of pcDNA3.1 containing wild-type *GABRB1* (WT- *GABRB1*) or variant *GABRB1* (MUT-*GABRB1*) plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to manufacturer's guide, respectively. RT-qPCR and Western blot analysis validated the expression of both mRNA and protein after 72h transfection.

Total RNA from HEK293 cells was extracted using TRIzol RNA reagent (Invitrogen), and 1 μg of RNA was prepared for reverse-transcription into cDNA using a PrimeScriptRT kit (TaKaRa, Shiga, Japan). The expression of *GABRB1* mRNA were analyzed by qPCR using the cDNA as template and SYBR qPCR Master Mix (Sparkjade, Jinan, China) on a Roche 480 Real-time Fast PCR System. The $2^{-\Delta\Delta Ct}$ method was performed to determine expression, with *GAPDH* as the internal control. The primers used in the qPCR are presented in Supplemental Table S1 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=175*).

Total protein was isolated from HEK293 cells in RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) and quantified using a BCA Protein Quantification Kit (Vazyme, Nanjing, China). The proteins were then separated by 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene fluoride membranes (Invitrogen). The membranes were blocked with 5% skimmed milk for 1 h and then incubated overnight at 4°C with primary antibodies against GABRB1 (ab16703; 1:1000; Abcam) and GAPDH (ab9485; 1:10000; Abcam). The membranes were then probed with a horseradish peroxidase conjugated secondary antibody (bs-40296G-HRP; 1:10,000; Bioss) for 1 h at room temperature. The protein bands were visualized using an ECL kit (Vazyme) and quantified using ImageJ software.

2.5. Statistical analysis

The results are presented as the mean ± standard error of the mean. Statistical comparisons were made with one-way ANOVA and the Tukey multiple-comparison tests using GraphPad Prism software, version 7.0 (GraphPad Software Inc., San Diego, CA, USA) to identify significant differences. *P* values lower than 0.05 were considered statistically significant (* represents $P < 0.05$, ** represents $P < 0.01$). All experiments were performed at least three times.

3. Results

3.1. Clinical history of the proband

The proband was a 7-year-old girl of normal height and weight at birth. She was the first child of a non-consanguineous couple of Chinese ancestry. Her family history was unremarkable. The child was born at 41+1 weeks gestation by vaginal delivery. The birth weight was 3,800 g, and the Apgar score was 10 at 0 min, 10 at 5 min and 10 at 10 min according to the standard (the reference population was Asian and Pacific Islander from the National Institute of Child Health and Human Development Fetal Growth Study) (*15*).

The patient had suffered from refractory epilepsy and developmental delay since the age of 2 months. She experienced recurrent convulsions 2 months after birth, characterized by loss of consciousness, clenched teeth, both eyes rolling or staring, facial cyanosis, limb shaking or stiffness, and urinary and fecal incontinence. Pinching therapy had a relieving effect on the condition in the initial stage. An initial diagnosis of hypocalcemia convulsion was made and she received calcium supplementation treatment, but there was no significant improvement in seizure control. Physical examination at the initial diagnosis showed clear consciousness and good mental health. The girl's head was free from deformities and she was sensitive to light. The patient had no resistance in the neck but muscle strength and tension of the limbs were normal. Biochemical examination was generally normal. EEG showed abnormalities with partial electrical seizures originating from the right central and middle temporal regions (Figure 1A).

When the patient was 5 months old, she experienced epileptic relapses with frequent blinking, gnathospasmus, purple lips, clenched hands, and an inability to exhale. Treatment with levetiracetam was initiated, with no improvement in seizure control. Consequently, several different anti-epileptic drugs were administered, including phenobarbital, lysine inositol, vitamin B12, and vitamin B6. Laboratory investigations, including humoral immunity, liver and kidney function, biochemistry, myocardial enzymes, and routine blood parameters, showed hepatic dysfunction. The patient also received new drug treatments, including oxiracetam, ribonucleic acid for injection, and reduced glutathione tablets. EEG examination showed abnormalities, and focal seizures in the right posterior temporal area were detected seven times on waking up and during sleep (Figure 1B). Results from cerebral magnetic resonance imaging (MRI) at the age of 5 months were normal (Figure 1C).

Abnormal EEG was observed at 9 months old. The background brainwaves were mainly characterized by medium to high amplitude irregular fast rhythms, with a high number of high amplitude spike waves, spike rhythms, and delta rhythms distributed asynchronously and asymmetrically in the left and right hemispheres of the brain (Figure 1D). EEG examination at 27 months of age showed an asymmetric frontal background rhythm (Figure 1E). EEG details at different ages are summarized in Supplemental Table S2 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=175*). Assessment of development at 27 months of age showed that the developmental age of each functional area of the patient was significantly lower than the actual age, especially in terms of adaptability, fine motor skills, and personal social interaction. Their developmental quotient was severely lower than the normal level (Table 1).

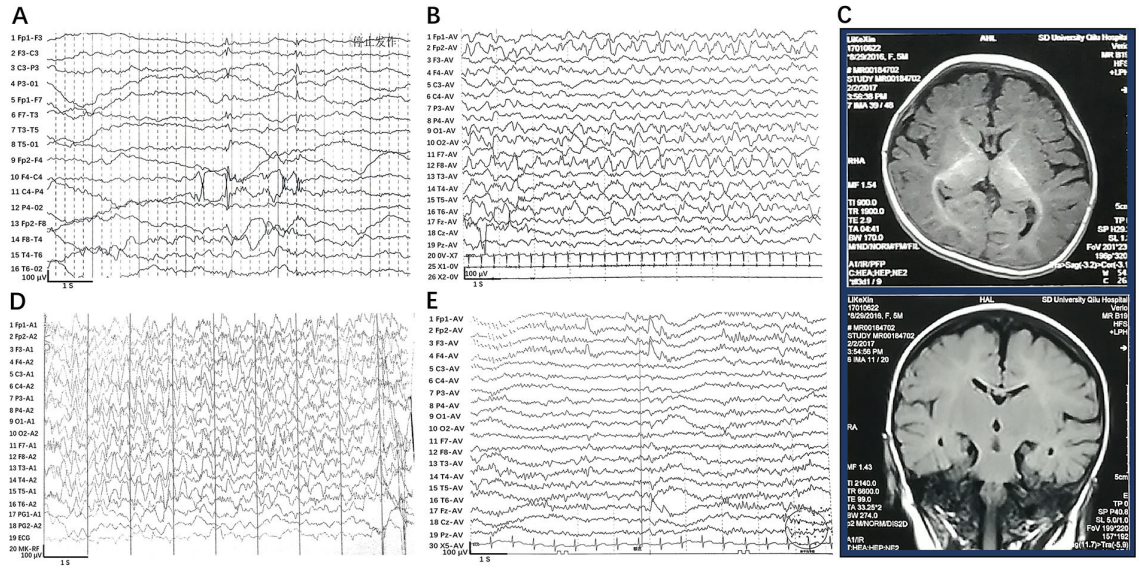

Figure 1. EEG and brain MRI features. (**A**) EEG of the patient at age 2 months was abnormal, with partial electrical seizures originating from the right central and middle temporal regions. (**B**) EEG of the patient at age 5 months showed focal seizures in the right posterior temporal area. (**C**) MRI of the patient at the age of 5 months showed no abnormalities in the brain. (**D**) EEG performed at the age of 9 months showed high amplitude spike waves and spike rhythms. (**E**) EEG of patient at age 27 months showed an asymmetric frontal background rhythm.

At the most recent follow-up, the patient was 7 years old, 115 cm tall, and weighed 17 kg. The patient's symptoms were comprehensive developmental delay, with severe language and motor developmental delay, poor cognitive ability, ataxia, dyskinesia, and decreased overall muscle tone.

3.2. Identification and verification of *GABRB1* mutation

Four variants were identified in the proband by WES: *GABRB1* c.858_859delinsTT, p.286_287delIleSer (NM_000812.4); *KCNQ2* c.1764-5G>A, (NM_172107.4); *KIF7* c.175G>A, p.Val59Met (NM_198525.3); *KIF7* c.3964T>C, p.Ser1322Pro (NM_198525.3). Sanger sequencing confirmed that only the *GABRB1* variation (c.858_859delinsTT) was *de novo* (Figure 2). The other three variants were either found in the proband's mother or father. *GABRB1* c.858_859delinsTT was only identified in the proband and not in her parents.

The p.286_287delIleSer change in *GABRB1* results in replacement of the encoded amino acids, 286 methionine and 287 threonine, with isoleucine and serine, respectively. *In silico* analysis with Mutation Taster predicted the pathogenicity of the variant to be damaging. The variant was not found in the Human Gene Mutation Database (HGMD), ESP6500siv2_ALL, 1000 person genome (1000g2015aug_ALL) and dbSNP147 databases. The variation was classified as likely pathogenic following the American College of Medical Genetics and Genomics guidelines.

3.3. Multiple sequence alignment and 3D structure predication

Multiple sequence alignment analysis among human α1-6, β1-3, and γ1-3 $GABA_A$ receptor subunits (Figure 3A) showed the conserved residues altered by the *de novo* mutations in this study. The GABRB1 protein sequence from different species were aligned and the variant located at amino acid 286-287 of GABRB1 protein within a highly evolutionary conserved region of the subunit (Figure 2B). Phylogenetic analysis indicating that the GABRB1 is relatively conserved among species ranging from fish to humans (Figure 3C).

The 3D view of the GABRB1 protein is shown in Figure 4A, indicating the location of the identified pathogenic variants. The novel variant, p.286_287delinsIleSer, identified in this study is in the second helix of the β1-subunit's transmembrane region affecting transmembrane domains, which is important for the function of the receptor (Figure 4B).

3.4. Increased GABRB1 expression in HEK293 cells

The expression of both wild-type and mutant GABRB1

Table 1. Developmental tests of the patient at the age of 27 months

Test energy zone	DA (month)	DQ	Evaluation
Adaptability	2.33	9	Extremely severe developmental delay
Gross motor	7.93	29	Severe developmental delay
Fine motor	2.80	10	Extremely severe developmental delay
Language	7.23	27	Severe developmental delay
Personal Social	2.10	8	Extremely severe developmental delay

DA: developmental age; DQ: development quotient. (According to the standards of the National Health Commission of China)

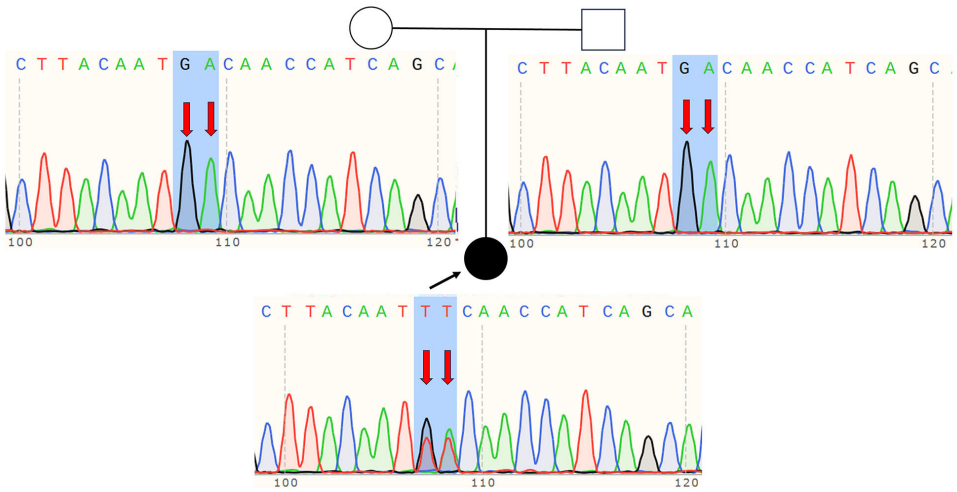

Figure 2. Sanger sequencing results of the proband and her parents. The single-nucleotide substitution is indicated by the red arrow.

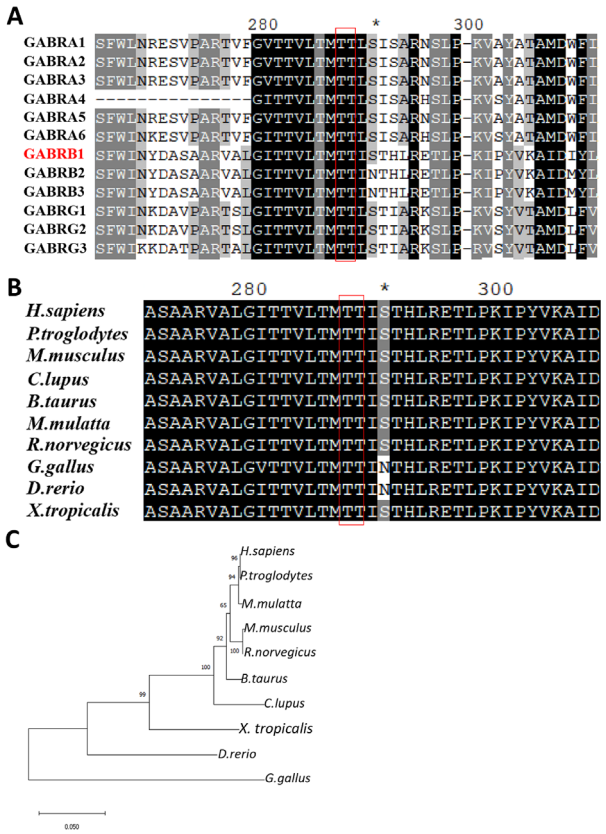

Figure 3. Multiple sequence alignment and phylogenetic analysis of GABRB1. (A) Multiple sequence alignments of human α1-6, β1-3, and γ1-3 $GABA_A$ receptor subunits. The residues highlighted in dark are conserved across all of the subunits. Conserved amino acids are indicated in dark (=100%) and light grey (≧75%) area. **(B)** The variant identified in this study (shown in red box) affected amino acids that are highly conserved from fish to humans. **(C)** Phylogenetic tree of the GABRB1 from different species. Branch confidence levels are built on 1000 bootstrap replicates.

are higher than that of control, referring to the success transfection and expression of wild-type and mutant GABRB1. Significantly enhanced expression was observed in the mutation of p.286_287delIleSer than that of wild-type GABRB1 (Figure 5A). Western blot revealed significant increase of GABRB1 expression level in mutant, which is consistent with the RNA expression (Figure 5B-C). Taken together, these results further confirmed that p.286_287delIleSer variation is a functional mutation. However, the molecular mechanisms still needs to be further explored.

4. Discussion

Epilepsy is a paroxysmal brain disorder that results from an imbalance between neuronal excitation and inhibition. Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the brain and plays an important role in the occurrence and development of epilepsy. Abnormalities in all aspects of GABA metabolism, including GABA synthesis, transport, genes encoding GABA receptors, and GABA inactivation, may lead to epilepsy. *GABRB1* encodes a subunit of one of the GABA receptors and is associated with epilepsy. Heterozygous variations in *GABRB1* on chromosome 4p13 can cause DEE45. Four DEE45 patients with heterozygous variations in *GABRB1* have been identified. In the study, we described a fifth DEE45 patient with a *GABRB1* variation, which is also the first DEE45 case in China.

The Epi4K Consortium and Epilepsy Phenome/Genome Project identified a single *de novo* heterozygous missense variant in *GABRB1* (c.737T>C, p.F246S) in 2013 (*2*). They reported a 4.5-year-old boy with epileptic

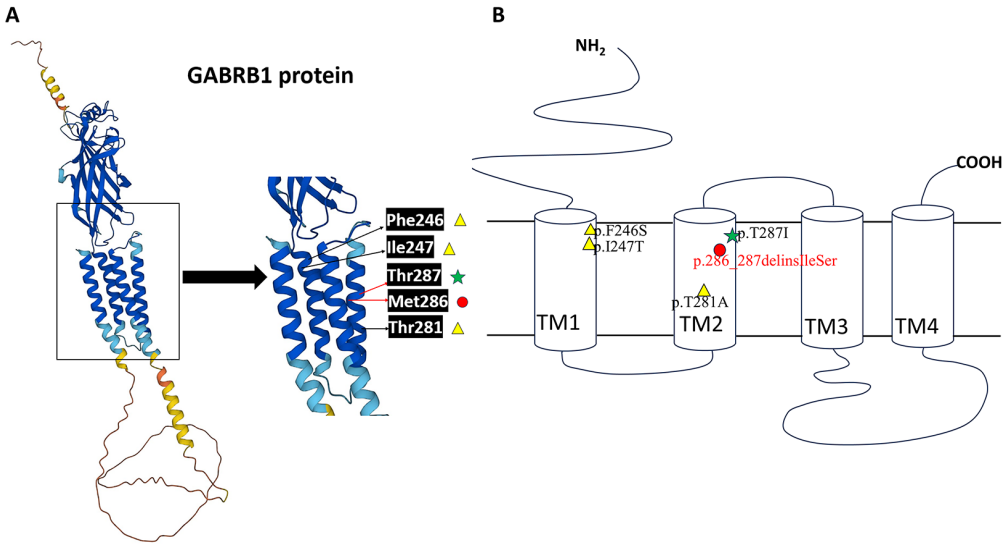

Figure 4. Location of the de novo mutations of *GABRB1* found in DEE45 patients. (A) 3D structural model of the GABRB1 protein (AlphaFold) indicating the positions of the identified pathogenic variants. **(B)** The p.F246S and p.M247I variants were in the TM1 domain; and the p.T281A, p. 286_287delinsIleSer, and p.T287I variants were in TM2 domain. (TM1-TM4) of GABAA receptors were predicted based on the method of Ernst *et al* (*19*). TM, transmembrane-spanning domain. Red circles indicate the variant (p. 286_287delinsIleSer) identified in this study, yellow triangles indicate previously reported variants (p.F246S, p.M247I and p.T281A), and the green star indicates the variants identified in this and previous studies (p. 286_287delinsIleSer and p.T287).

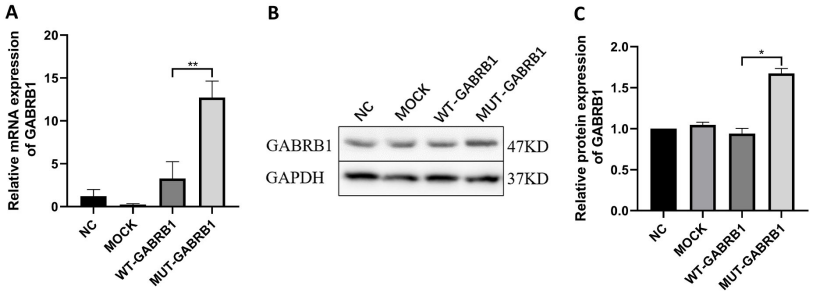

Figure 5. qPCR and Western blot of wild-type and mutant GABRB1 in HEK293 cells. Wild-type (WT) or mutant (MUT) GABRB1 was overexpressed in HEK293 cells. **(A)** mRNA was extracted from cells and quantified using RT-qPCR to measure GABRB1, relative expression of GABRB1 of wild-type is lower than mutant. **(B)** Western blotting showing expression levels of GABRB1 wild-type and mutant. **(C)** The intensity of the bands was quantified by densitometry. Results are expressed as fold-change relative to NC group. Data were normalized to GAPDH. **p* <0.01, ***p* <0.001. NC, negative control; MOCK, transfection reagent control.

encephalopathy. The patient had seizure onset at age 12 months and showed developmental regression at age 35 months. EEG showed hypsarrhythmia. He had global developmental delay, hypotonia, ataxia, cortical visual impairment, and a thin corpus callosum. Janve *et al*. published functional studies on this variant in 2016. The variant is reported to change the kinetic properties of the channel in HEK293 cells, resulting in a net loss of GABAergic inhibition (*16*).

In 2016, Lien *et al*. reported a 32-month-old boy with severe developmental delay and hypotonia who developed refractory epilepsy at age 3 months (*17*). Brain MRI of this patient was normal. This was the second case of DEE45 with a *de novo* heterozygous missense variation in GABRB1 (c.860C>T, p.T287I).

Burgess *et al*. (2019) reported a 2-year-old girl with DEE45 who carried a *de novo* heterozygous missense variation in *GABRB1* (c.740T>C, p.I247T). She experienced the onset of focal and tonic seizures at 4 months of age with cessation of seizures at about 2 years of age. She had profoundly impaired intellectual development with hypotonia, dysmorphic features, and progressive cerebral and white matter atrophy on brain imaging (*1*).

Monfrini *et al*. (2023) described the clinical and genetic findings of a 21-year-old woman with DEE45 carrying a novel *de novo* GABRB1 variation (c.841A>G, p.T281A) which was the fourth report of a DEE45 patient (*18*). This patient presented at birth with hypotonia and focal apneic seizures evolving in a phenotype of epilepsy of infancy with migrating focal seizures that were refractory to anti-seizure medications. Epileptic spasms that were partially responsive to steroid therapy appeared in the second year of life. Acquired microcephaly, profound mental retardation, and tetraparesis became evident with development. During childhood and adolescence, the epileptic phenotype evolved towards Lennox-Gastaut syndrome.

The novel mutation (p.286_287delinsIleSer) reported in our study is localized in the proximity of the previously described *GABRB1* pathogenic variant, p.T287I (Lien *et al*. in 2016), which affects the same alpha-helical transmembrane domain. In addition, methionine and threonine in this position are phylogenetic conserved. All above five *GABRB1* pathogenic variants affect transmembrane domains of the GABRB1 protein, possibility indicating a common molecular pathogenic mechanism causes of DEE45.

More noteworthy, however, is that through comparative analysis of these five DEE45 cases, we found that the brain MRI results of patients with p.F246S, p.I247T and p.T281A variants all showed a thin corpus callosum or white matter lesions. However, the brain MRI of the patient in this study (with a p.286_287delinsIleSer variant) and of a previously reported patient with a p.T287I variant were normal. Moreover, these two patients are similar in terms of age of onset. The overall severity of symptoms is also relatively mild compared with that in patients with the other three variants (p.F246S, p.I247T and p.T281A).

Taken together, this study reports the first case of DEE45 in China. Relationship between genotype and phenotype of DEE45 were overviewed through case analysis. The study also provides further clinical and molecular evidence for exploring the role of GABRB1 in this disease.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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