A *GABRB3* mutation (c.5G>A, p.Trp2*) in twins with generalized epilepsy with febrile seizures: A case report

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Abstract. In children aged 3 months to 6 years, convulsions that occur when the body temperature reaches >38˚C are referred to as febrile seizures (FS), provided that central nervous system infections and other metabolic etiologies are excluded. Generalized epilepsy with FS plus (GEFS+) refers to children who continue to suffer from FS after the age of 6 years with or without generalized and partial epileptic seizures. It is characterized by incomplete penetrance and genetic heterogeneity. In recent years, genetic techniques have undergone advancements, such that multiple genes can now be identified in clinical screenings, providing assistance in the diagnosis and delineation of diseases. Evidence suggests that mutations in a protein coding gene, γ-aminobutyric acid type A receptor β3 subunit (*GABRB3*) may be associated with GEFS+. The present case reports female twins with febrile seizures that typically occurred after fever, with seizures still occurring after the age of 6. Their growth and development were uneventful, with their laboratory, imaging and electroencephalography results almost within normal limits. After inquiring their medical history, it was found that the father of the twins had similar seizures when he was young. Next, peripheral blood samples were collected from the twins and their parents for whole-exome sequencing. The gene sequencing results showed that the twins and their father had a *GABRB3* nonsense mutation (c.5G>A, p.Trp2*), which causes translation to terminate prematurely at the second tryptophan position. After consulting the literature, it was considered that they may have *GABRB3* mutation-associated GEFS+. After treatment with levetiracetam, the seizures in children have been effectively controlled and their growth and development have not been affected, which is within the normal range.

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Introduction

Febrile seizures (FS) are the most common cause of convulsive events during childhood. In addition, they also account for the majority of seizures in children, occurring in 4-10% of all cases (1,2). A number of children will continue to experience frequent bouts of FS after the age of 6 years, which is referred to as FS+. By contrast, generalized epilepsy with FS+ (GEFS+) is an epilepsy syndrome that is typically diagnosed in families as a whole, which was included in the classification of epilepsy syndromes by the International League Against Epilepsy (3) in 2001. This condition was first reported in a study by Berkovic and Scheffer in 1997 (4,5). Previous studies have shown that genetic factors are closely associated with GEFS+. Additionally, the incidence rate of FS is different among different ethnicities, with an incidence rate of 2-5% in Europe and the Unites States, but an incidence rate of 5-14% in Asian countries (6-8). The risk of FS in individuals that have siblings with FS has been reported to be 9-22%, where the concordance rate is higher in identical twins compared with in fraternal twins. Furthermore, 25-40% children with FS were found to have a positive family history of FS. However, to the best of our knowledge, studies assessing the association between the incidence rate of GEFS+ and age remain scarce.

γ-aminobutyric acid (GABA) type A ($GABA_A$) receptors primarily mediate rapid inhibitory neurotransmissions that occur in the brain. They are mostly formed by the co-assembly of two α, two β and one γ subunits (9). Amongst these, the β3 subunit is encoded by the γ-aminobutyric acid type A receptor β3 subunit (*GABRB3*) gene, which is located on chromosome 15q11.2-q12 (10-12). $GABA_A$ receptors are mediators of the

rapid inhibition of synaptic ligand-gated chloride channels in the central nervous system. When GABA binds to $GABA_A$ receptors at β3/α-specific binding sites at the interface between the subunits, the ion channel opens. This causes an influx of chloride ions into the cell, decreasing neuronal excitability (13). Therefore, adequate expression of each subunit is important for the function of $GABA_A$ receptors. *GABRB3* is highly expressed during the early stages of embryonic brain development and is crucial for the assembly and transportation of GABA receptors upstream of stem cell differentiation (14). Gene mutations in *GABRB3* can result in changes to the function of the channel, leading to a decrease in GABAergic postsynaptic inhibition. This pathological consequence has been reported to associate with the heterogeneity of epilepsy (15). Recently, *GABRB3* mutations have been identified in patients with infantile spasms and Lennox-Gastaut syndrome. However, because of the rarity of *GABRB3*-associated GEFS+, the phenotype-genotype association between *GABRB3* mutations and GEFS+ remain to be fully elucidated. Since relevant cases remain limited and further studies are required (16).

Case report

At the age of 1 year and 8 months (January 2019), a female patient (child A; the younger individual of twins) was admitted to the Affiliated Hospital of Jining Medical University (Jining, China) with a 3-day fever and one occurrence of convulsions. The temperature of child A was 38.4˚C, who did not have a cough, sputum, abdominal pain, diarrhea, nausea or vomiting. The patient had one convulsion at the beginning of the illness, which manifested as a loss of consciousness, inability to respond to calls, an upturning of the eyes, clenching of the hands and rigidity of the limbs. It lasted for several tens of seconds before stopping without treatment. During the convulsion, the patient did not foam at the mouth, with no urinary or fecal incontinence. The past growth and development of child A were in line with normal ranges. The parents of the patient were healthy and did not have a consanguineous marriage. Furthermore, the parents of the patient and family members denied any history of major diseases. However, the father and twin sister of the patient had a history of similar febrile convulsions. On the basis of the medical histories and relevant auxiliary examination results from the twins, the twins were diagnosed with a clinical phenotype of FS syndrome (FS+), with seizures that were mainly in the form of cataplexy and focal seizures.

Results of a physical examination were as follows: Temperature, 36.8˚C; pulse, 108 beats/min; respiration, 28 breaths/min; weight, 15.5 kg; and height, 97 cm. The patient was alert, in no acute distress, had achieved age-appropriate developmental milestones, was well-nourished, and exhibited a ruddy complexion. There were no observable rashes on the hands, feet or buttocks. Child A had pharyngeal congestion, with thick respiratory sounds in both lungs, but dry or wet rales could not be heard. The heart sound were normal and crisp, where the abdomen was soft, without abdominal distension. The liver and spleen were not enlarged, whereas Brudzinski's, Kernig's and Babinski's signs were all negative. The results of relevant laboratory tests were within normal limits. From cranial MRI, the bilateral cerebral hemispheric structure was symmetrical and the brain white matter contrast was normal. However, a patchy foci of slightly high abnormal T2-fluid-attenuated inversion recovery signals were observed in the bilateral occipital lobes (Fig. 1). Abnormalities were not observed in the patient during electroencephalography (Fig. 2). In addition, abnormalities were not observed in the cranial MRI examinations of the mother (Fig. 3A), father (Fig. 3B) and the twin sister (child B; Fig. 3C) of child A.

Child A was diagnosed with 'epilepsy observation' from a referral hospital (specific details unknown) in December 2019, and immediately started to take 2 ml levetiracetam oral solution (100 mg/ml) twice daily. No seizures occurred during the treatment period. However, after stopping the medication for 2 days in June 2020, the patient had one seizure. Therefore, the patient continued to take 2 ml levetiracetam (100 mg/ml) twice daily. In July 2020, child A experienced a seizure after another fever, which was characterized by a trance-like consciousness, dazed eyes, staggering when walking and an inability to respond to calls. The seizure was also accompanied by tachypnea and bilateral manual automatisms, more prominent on the right side, followed by gradual assumption of a prone position. No clonic movements were observed during the ictal phase. The seizure lasted for ~5 min before it was relieved. Subsequently, in the outpatient department of the hospital, the medication was adjusted to 2.5 ml levetiracetam (100 mg/ml) twice daily, following which no further seizures were reported. In September 2022, the levetiracetam dosage was reduced by 0.5 ml every ~2 months. In April 2023, at which time the levetiracetam dosage was 0.5 ml in the morning and 1.0 ml in the evening), the patient had another fever (body temperature of 38.0˚C) and again experienced a seizure. After a consultation in our epilepsy clinic, it was recommended to increase the dosage of levetiracetam (100 mg/ml) back to 2.5 ml twice daily. Child A was followed up to the present day and was aged 6 years and 5 months at the last follow-up appointment (November 2023). Despite regular medication, the patient still experiences seizures, which manifest as trance-like consciousness, dazed eyes and an inability to answer calls. The seizures typically lasted 5-6 min before resolving.

Child B, the elder twin sister, had symptoms similar to FS. Fevers >38˚C occasionally induced convulsions, which manifested as a loss of consciousness, inability to respond to calls, staring eyes, cyanosis, limb convulsions and foaming at the mouth. These seizures lasted for 1-2 min before relieving. In January 2020, child B began oral treatment of 2 ml levetiracetam (100 mg/ml) twice daily. The patient discontinued the medication for 3 days during treatment and then continued oral administration, during which time there were no seizures. In May 2022, the patient began to reduce the dosage of levetiracetam (100 mg/ml) by 0.5 ml every ~2 months, before discontinuing the medication in December 2022. However, in the middle of December 2022, the patient experienced a fever that was induced by coronavirus-19 disease. Convulsions occurred twice when the body temperature of the patient reached 38˚C, with each seizure lasting for ~2 min at an interval of 8 h. In March 2023, convulsions again occurred as the result of a fever that was caused by influenza A infection. The patient was in a standing position and suddenly fell, which was followed by convulsions that lasted for 2-3 min before they were relieved. The patient then slept and woke up

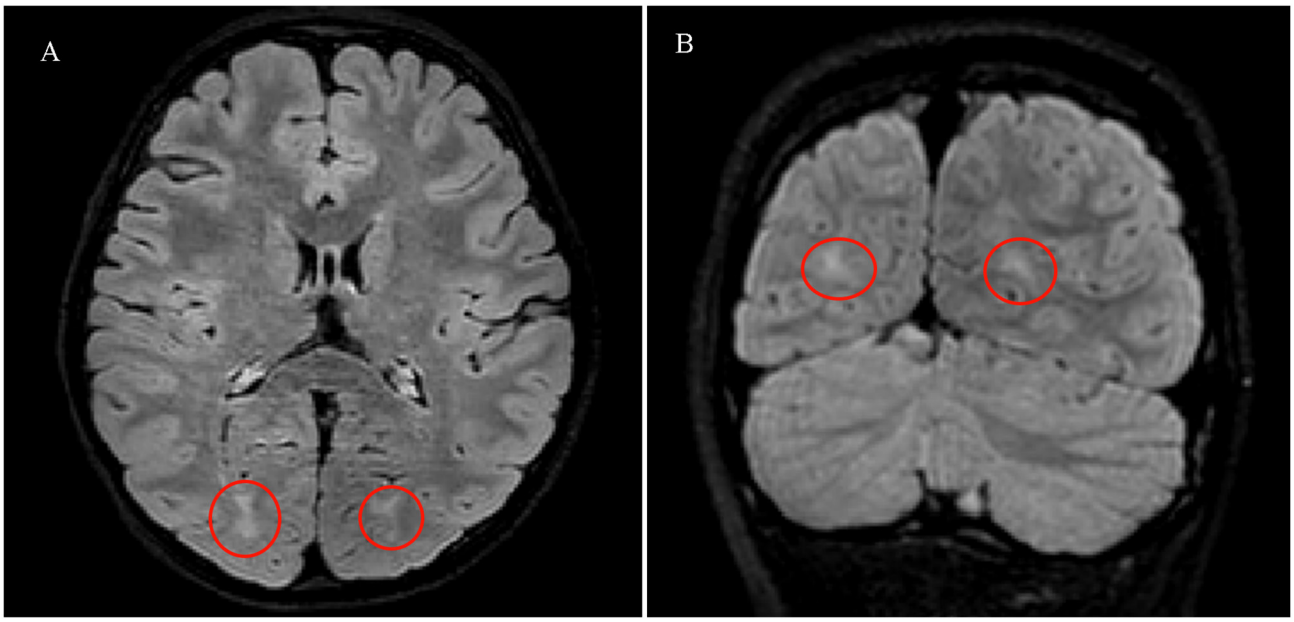

Figure 1. Cranial MRI of the patient. (A) axial view and (B) coronal view. The bilateral cerebral hemispheric structure was symmetrical and the brain white matter contrast was normal. However, patchy foci of slightly high T2-fluid-attenuated inversion recovery abnormal signals were observed in the bilateral occipital lobes (as indicated by the red circles).

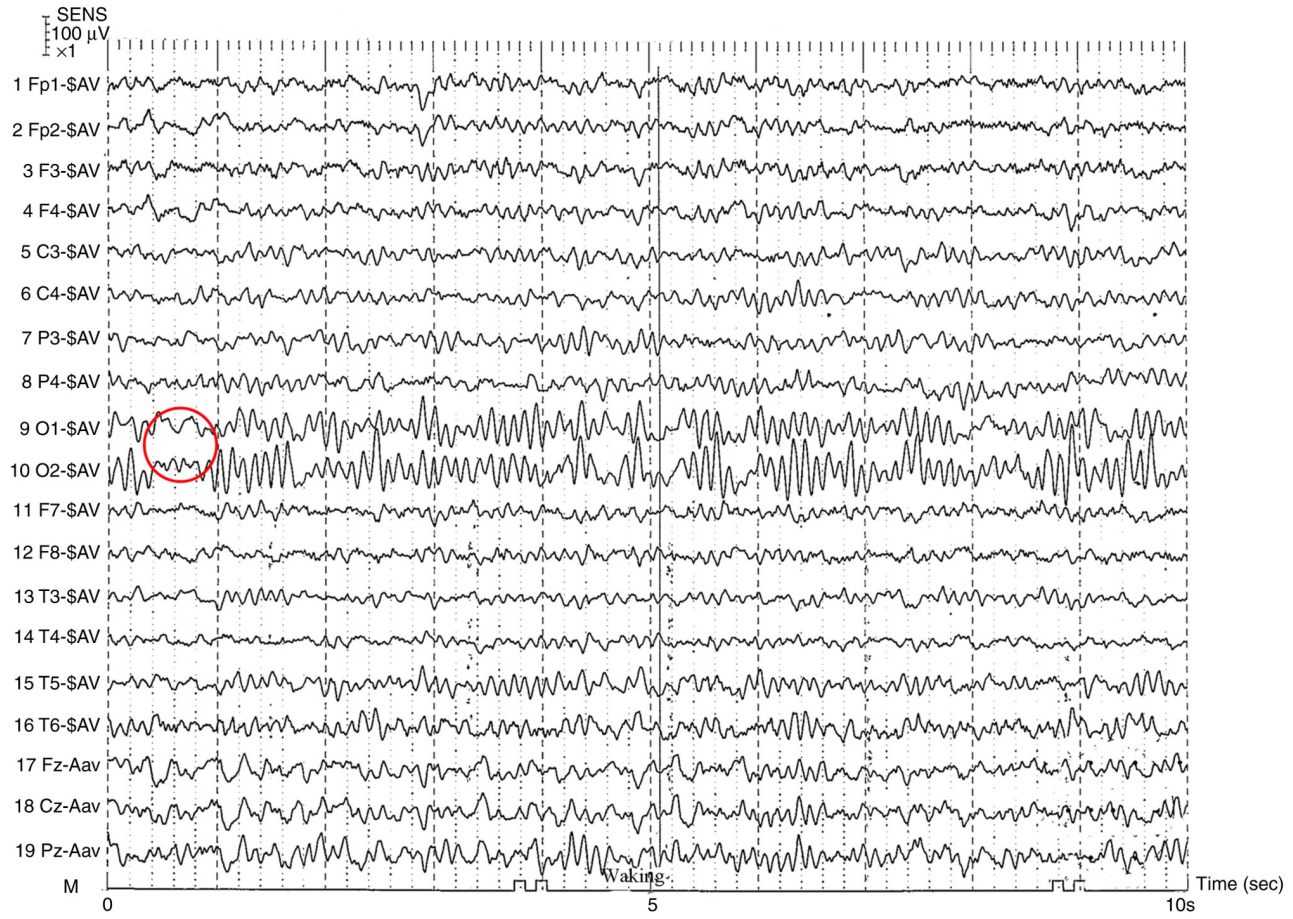

Figure 2. Electroencephalography of the patient. During the awake, quiet, and eye-closed state, bilateral occipital regions exhibited 10.0-11.0 Hz alpha rhythm at 40-60 μV, intermixed with sparse irregular waves. Activity was approximately symmetrical on the left and right sides, indicating that the regulation of amplitude modulation was acceptable. With the eyes closed α inhibition and appearance was observed. Peak, spindle and slow waves were observed during the sleep period, and the left and right sides were generally symmetrical, indicating the absence of a sleep cycle disorder. SENS, sensitivity; Fp1, frontopolar left; Fp2, frontopolar right; F3, frontal left; F4, frontal right; C3, central left; C4, central right; P3, parietal left; P4, parietal right; O1, occipital left; O2, occipital right; F7, anterior temporal left; F8, anterior temporal right; T3, mid-temporal left; T4, mid-temporal right; T5, posterior temporal left; T6, posterior temporal right; Fz, frontal midline; Cz, central midline; Pz, parietal midline; -SAV, scalp average reference; -Aav, auricular average reference; M, ground electrode.

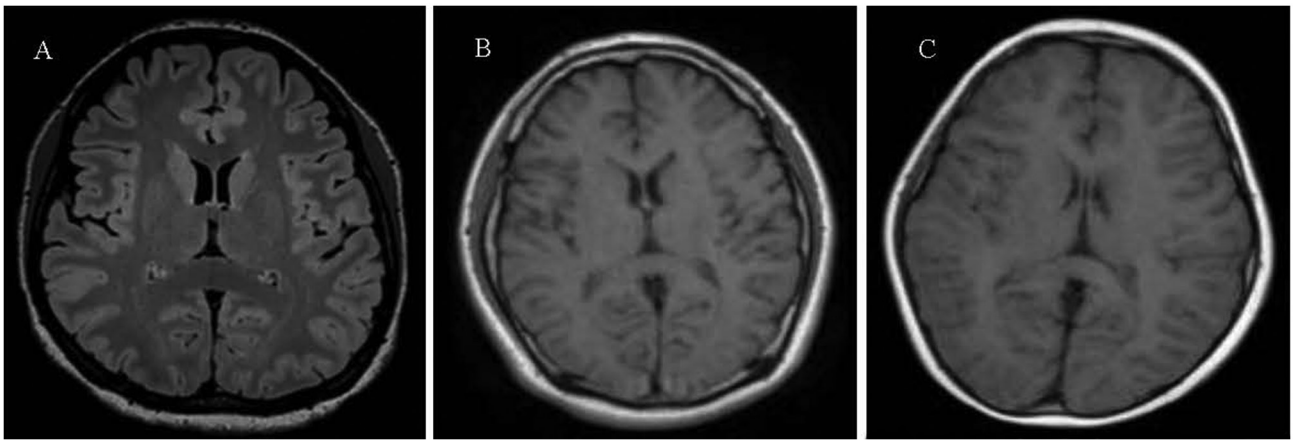

Figure 3. Cranial MRI of the family of the patient. Symmetrical bilateral cerebral hemispheric structures and normal brain white matter contrasts were observed in (A) the mother, (B) father and (C) elder twin sister of the patient, without any abnormal signals.

with no abnormalities. In September 2023, the patient again developed a fever, which reached 39.4˚C and convulsions occurred (the specific details are unknown). From December 2022 until October 2023, no medication was used. However, in October 2023, the patient came to our outpatient service and was prescribed 2.5 ml levetiracetam (100 mg/ml). At present, although the patient is maintained on 2.5 ml levetiracetam twice daily, the patient still experiences FS.

Neither child A nor child B received long-term systematic hospitalization treatment, meaning that they did not receive any specialist care. When the twins do not experience fever-associated convulsions, their quality of life was not affected and they exhibit regular growth and development.

The father of the patients also experienced FS during childhood, although the exact manifestations of the seizures were not recalled. However, the father did not take any antiepileptic drugs and does not currently experience seizures (as of November 2023, the individual was 38 years old.). The disease status of each family member is presented in Fig. 4.

In total, 4 ml venous blood of the proband and 2 ml blood samples of Child A's parents and sister were collected and sent to Beijing Kangxu Medical Laboratory for whole-exon detection. Genomic DNA was extracted from blood samples using the Qiagen FlexiGene DNA Kit (cat. no. 51206; Qiagen GmbH) and the genomic DNA quality control was performed. Agarose gel electrophoresis was used to analyze the degree of DNA degradation and whether there was RNA and protein contamination. Qubit 2.0 fluorometer was used to quantify DNA concentrations. The Covaris Ultrasonic DNA Fragmentation Instrument (Covaris LLC) was used to randomly interrupt fragments of genomic DNA with a growth of 180-280 bp using the SureSelect XT Library Prep Kit ILM (cat. no. 5190-8863; Agilent Technologies, Inc.). After repairing the end of DNA fragment and adding A-tail, DNA library was prepared by connecting splices at both ends of the fragment. The DNA library was amplified using the TransNGS Index Primers (384) Kit (cat. no. 3KI241; TransGen Biotech Co., Ltd.) according to the manufacturer's protocol: Initial denaturation was at 95˚C for 10 min; followed by 35 cycles of 30 sec at 95˚C, 30 sec at 60˚C and 45 sec at 72˚C, with a final extension at 72˚C for 5 min. The subsequent adaptor-specific primers were employed for the amplification of the DNA library: Forward, 5'-GGGGAGTCAGGTGCAAGAG-s-T-3' and reverse 5'-GAAGCGACAGTCACAACTTCC-s-T-3' (-s-represents a phosphorothioate bond).

A library with a specific index tag was hybridized in liquid phase with up to 500,000 biotin-labeled Agilent SureSelect Human ALL Exon V6 probes (cat. no. 5190-8863; Agilent Technologies, Inc.), before being captured using streptomycin beads. After PCR linear amplification, the library was inspected and sequenced. After the library construction is completed, use Qubit 2.0 fluorometer (Thermo Fisher Scientific, Inc.) was used for preliminary quantification, before Agilent 2200 (Agilent Technologies, Inc.) was used to detect the insert size of the library. The final concentration of the library was 95.1 ng/μl for Child A, 71.3 ng/μl for Child A's father, and 96.6 ng/μl for Child A's mother. Subsequently, NovaSeq6000 S4 Reagent Kit v1.5 (300 cycles; cat. no. 20012866; Illumina, Inc.) was used to perform double-ended sequencing on the NovaSeq 6000 (Illumina, Inc.) platform, with each end measuring 150 bp and an average sequencing depth of 100X and the data in fastq format was obtained.

The sequencing read was compared to the Human reference Genome (hg19 version) using the BWA tool (v0.7.15). The comparison results were converted into bam format for variation analysis. GATK (v3.6; https://www.broadinstitute.org) was used to detect single nucleotide mutations and small insertion loss variations. CODEX (v1.14.1; https://www.bioconductor.org/packages/release/bioc/html/CODEX.html), XHMM (v1.0; (https://zzz.bwh.harvard.edu/xhmm/index.shtml) and Kang Xu Capture sequencing copy number variation detection software V1.0) were used to analyze possible copy number variations. Gene-related annotation analysis was performed using the reference genome version GRCH37/Hg19 (https://www.ncbi.nlm.nih.gov/refseq), Ensembl (http://grch37.ensembl.org/Homo_sapiens/Info/Index) and UCSC (version GRCH37/Hg19 reference genome; https://genome.ucsc.edu) databases. The 1000G (2015 update; http://www.1000genomes.org), dbSNP (v150; https://www.ncbi.nlm.nih.gov/SNP) and ExAC (v0.3; ExAC is now in gnomAD; www.gnomad-sg.org) tools were used to annotate the frequency of variation in a population. PolyPhen2 (version 2; http://genetics.bwh.harvard.edu/pph2), SIFT (version 2; https://sift.bii.a-star.edu.sg) and MutationTaster

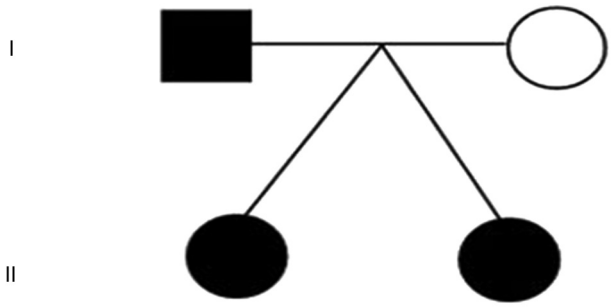

Figure 4. Pedigree diagram of the four family members. This diagram indicates that the father and twins were affected, but the mother was not. Squares, male; circles, female; shaded, individual was affected by the disease; non-shaded, individual was not affected by the disease.

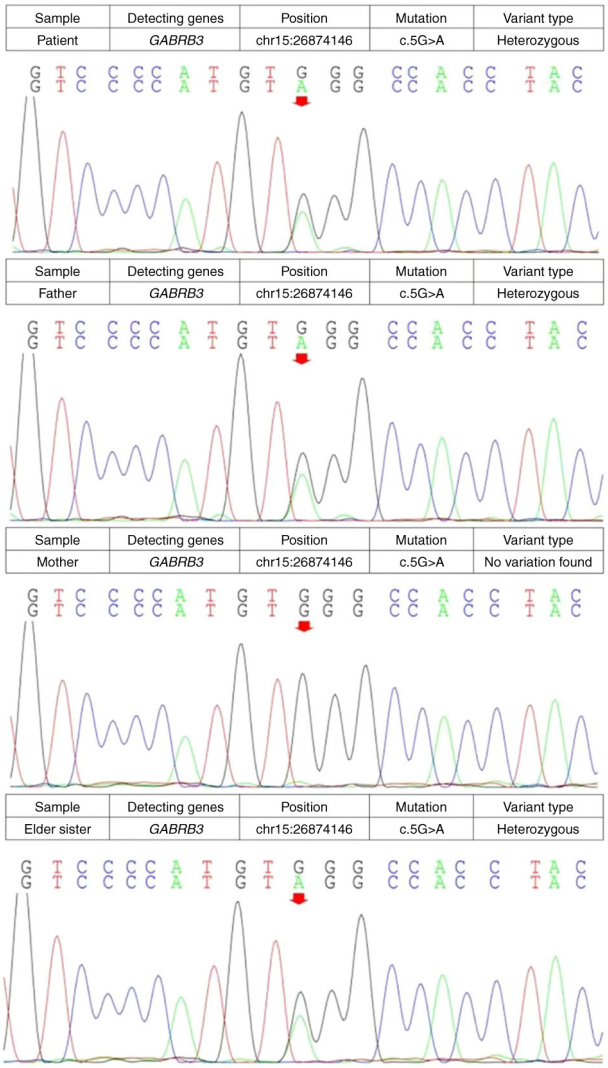

Figure 5. Sanger sequencing indicated that the patient had a nonsense mutation in the *GABRB3* gene. A heterozygous nucleotide variation, c.5G>A, was identified in the patient. The twin sister and father of the patient also had the c.5G>A *GABRB3* mutation. (A) patient with *GABRB3* gene mutation. (B) Patient's father with *GABRB3* gene mutation. (C) Patient's mother with no variation found in *GABRB3*. (D) Patient's sister with *GABRB3* gene mutation.

(NCBI 37/Ensembl 69; http://www.mutationtaster.org) were used for protein gene locus mutation function damage prediction. OMIM (https://www.omim.org/), HGMD (http://www.hgmd.org) and ClinVar annotations (https://submit.ncbi.nlm.nih.gov/clinvar/) were used to make disease-related annotations associated with disease. Sites were classified using the American Society for Medical Genetics and Genomics (ACMG) variation scale with the Society for Molecular Pathology system (pathogenic, possibly pathogenic, of uncertain significance, possibly benign and benign). The annotation results are filtered according to the characteristics of the mutation sites in the database and the variation that may be associated with the disease is retained.

Subsequently, candidate mutation sites were investigated using Sanger sequencing. The human genome GenBank database-gene sequences, the Primer design website design primers and Primer Z (http://genepipe.ncgm.sinica.edu.tw/primerz/primerz4.do). PCR amplification was then performed, before the ABI 3730 generation sequencer (Thermo Fisher Scientific, Inc.) was used for the sequencing of the amplification products. The obtained data were visualized using the Chromas software (V2.6.6) and compared with the results of whole exome sequencing.

A nonsense mutation in *GABRB3* (c.5G>A, p.Trp2*) was detected in both of the patients and their father. This mutation was present in the father of the patients but not in their mother, suggesting that it was of paternal origin (Fig. 5). c.5G>A, p.Trp2* is Exon 1, the second amino acid encoded by this gene changes from tryptophan (TRP) to a stop codon, causing protein translation to terminate early.

The American College of Medical Genetics and Genomics criteria indicated very strong pathogenic evidence (PVS1_VeryStrong; PS4). 'PVS1_VeryStrong' signify the pathogenic mechanism of a gene is through the loss of function, non-sense mutations, frameshift mutations, ±1 or 2 position splicing mutations, start codon variations, or single or multiple exon deletion mutations on the gene. Strong pathogenic evidence 'PS4' means that the frequency of mutations occurring in the relevant patient population is notably higher compared with that in the control group.

Discussion

GEFS+ is an ion channel disease for which the pathogenesis has not been fully elucidated, although previous studies have revealed a genetic susceptibility. The mechanism of GEFS+ inheritance is complex, although the majority of the cases autosomal dominant. This condition is characterized by phenotypic and genetic heterogeneity, with diverse clinical phenotypes (17). Numerous candidate genes have been reported to be associated with FS or FS+, including GABA receptor-associated genes (such as *GABRA1*, *GABRB3*, *GABRD* and *GABRG2*), voltage-gated sodium ion channel-associated genes (*SCN1A*, *SCN1B*, *SCN2A* and *SCN9A*) and transport-associated genes, such as *SLC12A5* and *SLC32A1* (18-21). This type of genetically associated FS+ is known as GEFS+, which is a common familial epilepsy syndrome. Therefore, a history of FS and FS+ in family members is important for the diagnosis of GEFS+ in an individual.

GABA is one of the most important inhibitory neurotransmitter in the brain. It serves an important role in epilepsy onset and development (22). Abnormalities in various aspects of GABA metabolism can lead to seizures. Under normal circumstances,

GABA maintains a balance of neurotransmitter content in the brain through the glutamate/GABA/glutamate cycle (23), to ensure its inhibitory effects are exerted. When genetic alterations result in the inability of mutated subunits to pair with normal subunits, GABA receptor assembly becomes impaired to obstruct receptor function (14). This frequently results in epileptic syndromes with different clinical phenotypes, either due to downregulated surface receptor expression or decreased magnitudes of GABA-induced inhibitory potentials (22,24). All GABA-related receptors have similar structures, with four transmembrane (TM) domains (TM1 to TM4) and a long extracellular N-terminal domain. Ion channel pores are formed by the TM2 of each subunit. The pathogenesis of *GABRB3* mutations is due to the function of the protein domain (11,12). Regardless of the receptor, mutations located in genes encoding the TM regions of a receptor subunit are associated with more severe phenotypes, whereas mutations in the N-terminal appear to be associated with milder phenotypes. Rather than being associated with the mutated gene, the clinical phenotypes of *GABRB3*-related mutations are more likely associated with the location of the mutation within the structure of the protein (25-27).

GABRB3 has nine exons in total, is ~230 kb in length and is highly expressed in various regions of the brain. The β3 subunit encoded by this gene is the earliest β subunit to appear in the embryonic brain, where its expression level in the perinatal brain is typically 150% higher compared with that in the adult brain (24). Data from previous studies using rodent models suggest that β3 subunits are expressed during development, but that the expression of β3 declines postnatally (28,29). As a $GABA_A$ receptor subunit, β3 serves a central role in $GABA_A$ receptor assembly and trafficking to the cell surface (30). Unlike other $GABA_A$ receptor subunits, which require assembly with accompanying subunits for surface localization, the β3 subunit can be transported to the cell surface and form homologous pentamers when expressed alone because it contains four amino acids (arginine 180, glutamate 179, lysine 173 and glycine 171). This is specific to the β3 subunit, suggesting its unique ability for homologous oligomerization and membrane targeting (14). Considering the etiology of neurodevelopmental disorders, *GABRB3* is abundant in the early brain and has been reported to induce stem cell differentiation (31). Furthermore, β3 subunit-dependent phosphorylation mediates $GABA_A$ receptor accumulation and the immobilization of the inhibitory synaptic scaffold protein gephyrin at synapses. These events are crucial for the long-term potentiation of inhibition and may modulate network excitability (32). In addition, β3 subunit-adaptor protein 2 interactions stabilize $GABA_A$ receptors at endocytic zones and may serve a role in regulating the number of synaptic receptors during inhibitory synaptic plasticity (33). These aforementioned findings indicate that β3 subunits serve a role in regulating synaptic strength and brain development. In the present case, a *GABRB3* mutation was identified, which may cause changes to the structure and function of the β3 subunit, resulting in it being unable to pair and bind with other subunits normally, thereby reducing the expression levels of the $GABA_A$ receptor. This may lead to the decreased postsynaptic inhibitory effect of GABA, preventing it from exerting its inhibitory neurotransmitter effects and affecting the excitability of the neural network, increasing the risk of seizures.

Exons 1 and 1A of *GABRB3*, along with exons 2-9, produce selective mRNA transcripts that two specific signal peptide sequences are derived from (34). These sequences encode two mature polypeptides that have slight variations in the N-terminal residues and produce different signaling peptide sequences. Therefore, a mutation may lead to structural modifications of the β3 subunit of $GABA_A$ receptors (35). Previous studies of GABA gene-associated childhood catatonic epilepsy have identified various missense mutations, such as (c.31C>T; p.Pro11Ser), (c.44C>T; p.Ser15Phe) and (c.94G>A; p.Gly32Arg), all of which are located in exon 1A of *GABRB3*. These mutations presumably cause the hyperglycosylation of $GABA_A$ receptor β3 subunit proteins, causing a decrease in GABA-evoked currents, leading to seizures (16). In the present case, it was hypothesized that the identified mutation (c.5G>A, p.Trp2*) may alter the structure of the $GABA_A$ receptors and lead to its dysfunction, resulting in an impaired GABA-mediated inhibition in the brain and the development of GEFS+ in children.

The first case of epilepsy associated with a *GABRB3* mutation was reported in 2008 (35). An increasing number of reports have shown a strong association between *GABRB3* mutations and epilepsy (36,37). The majority of known cases of *GABRB3* mutations are in children with early onset epileptic encephalopathy (34). Furthermore, Tanaka *et al* (35) previously reported that *GABRB3* mutations are associated with childhood absence epilepsy. *GABRB3* mutation-associated epileptic seizures can take a variety of forms, including generalized tonic-clonic, tonic, infantile spasms, myoclonic and atonic (16,35,38). The majority of such seizures tended to have a febrile trigger in affected patients. Patients tend to exhibit different degrees of delays in language, motor and intellectual development. In addition, a number of patients have demonstrated various mental/behavioral disorders, such as restlessness, autism spectrum disorder, attention deficit hyperactivity disorder or aggressive behavior. In terms of clinical phenotypes, *GABRB3* variant-associated epilepsy can manifest as multiple forms of epileptic syndromes (16,35,38). The prognosis is generally favorable for catatonic epilepsy, FS and additional febrile convulsions. However, prognosis is poor for patients with Lennox-Gastaut syndrome, West syndrome and infantile spasms, which are frequently associated with parenchymal damage). In addition, a number of children with epileptic phenotypes of FS with additional symptoms and catatonic epilepsy have normal intellectual, motor and language development (16,39). Upon examination using imaging, the majority of patients show a normal MRI of the cranium, although a small number of patients will show abnormalities, such as reduced myelin sheaths, multiple gyrus malformations, cerebellar hypoplasia, abnormalities of the corpus callosum or severe diffuse cerebral atrophy (16). In the present case, the twins and their father had a *GABRB3* mutation that was not identified in the mother. Brain MRI scans of this family revealed no notable abnormalities, further indicating that patients with *GABRB3* mutation-associated FS have a mild phenotype and favorable prognosis.

In the present case report, the twins presented with seizures in early childhood, where the majority of seizures occurred after a fever of >38˚C. The seizures were characterized by a loss of consciousness, staring, cyanosis, staggering and convulsions of the limbs with or without foaming at the mouth, which lasted for a period of a few sec to lasting seconds to minutes. This was confirmed from the medical histories

of the twins and the results of auxiliary examinations. Upon follow-up (November 2023), it was revealed that the twins still experienced seizures despite being aged >6 years. A nonsense mutation in *GABRB3* (c.5G>A, p.Trp2*) was detected in the twins and their father, which revealed a familial predisposition, with a history of FS in the father. After performing a literature review, it was considered that this gene may be associated with the seizures in the twins (they may have *GABRB3* mutation-associated GEFS+). However, in the present case, the twins and their father had different seizure patterns. Since *GABRB3* expression is high in the embryonic brain and gradually decreases in adulthood, although the mutated genes were the same, the associated clinical phenotypes may differ because of the differences in expression levels among different individuals. However, notable genotype-phenotype associations have previously been reported in terms of the localization of variants within the protein domain of $GABA_A$ receptor subunits. Additionally, an in-depth historical and literature review has corroborated that the prevalence of GEFS+ may be age-independent. In the present case, the seizures of the twins may have decreased compared with before. The reasons for this may be as follows: i) The twins now regularly use medication and the drug-controlled effect is satisfactory; ii) the frequency of the fevers that the twins experience has decreased compared with before, thereby reducing the likelihood of seizures; and iii) compared with infancy, the expression of *GABRB3* is reduced in adulthood, meaning that the human body may be less affected by its level of expression.

Therapeutically, generally acceptable seizure control has been reported in cases of *GABRB3* mutation-associated epilepsy after treatment with sodium valproate, perampanel and clonazepam (40). The method of drug selection for GEFS+ is mainly based on the clinical phenotype and pathogenesis. Valproic acid and carbamazepine are typically used as first-line drugs, though newer antiepileptic drugs, such as lamotrigine and levetiracetam, are becoming more widely used because of their higher safety profiles and milder side effects. Amongst these aforementioned new drugs, levetiracetam selectively inhibits high-voltage-activated calcium channels and reduces calcium release from intraneural stores. Furthermore, it has no inhibitory effects on simple seizures induced by convulsant stimulation and shows only weak activity in maximal stimulation and threshold tests (41). *In vitro*, levetiracetam has been reported to exert no effects on neuronal voltage-gated sodium channels or T-type calcium currents, nor did it directly predispose cells to GABAergic neurotransmission. However, it did counteract the activity of negative regulators of GABA-activated currents and glycine-gated currents, whilst partially inhibiting N-type calcium currents in neuronal cells (40). Furthermore, levetiracetam has been documented to inhibit hippocampal epileptiform bursts of discharge without affecting normal neuronal excitability, suggesting that it may selectively inhibit epileptiform bursts of discharge, super-synchronization and seizure propagation (42). In the present case, levetiracetam was administered to the twins for seizure control with favorable outcomes. However, they both had difficulties in discontinuing the drug. At the latest follow-up, they remain dependent on levetiracetam regularly for seizure control. Therefore, the present case may provide assistance to clinicians when treating children with GEFS+.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The novel c.5G>A (p.Trp2*) missense mutation in *GABRB3* was deposited in SRA (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1272306/; accession no. PRJNA1272306).

Authors' contributions

SL, RL and QK designed the study. YW, KF, SR, LW, JG and XC collected the data. SL, JL, RL and QL contributed to data analysis and interpretation. SL and RL drafted the manuscript. QK and RL contributed to the revision. SL and JL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present case was approved by The Ethics Committee of The Affiliated Hospital of Jining Medical University (approval no. 2023-09-C031; Jining, China).

Patient consent for publication

The patients' parents provided written informed consent for the publication of any associated data, as well as accompanying images and videos.

Competing interests

The authors declare that they have no competing interests.

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