Clinical Case Report

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Early onset epileptic encephalopathy with a novel GABRB3 mutation treated effectively with clonazepam

A case report

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Abstract

Rationale: Early onset epileptic encephalopathy (EOEE) is one of the most serious early onset epilepsies. The etiopathology of this condition remains unclear, and recent evidence indicated that gamma-aminobutyric acid (GABA) A receptor, subunit beta 3 (*GABRB3*) gene mutations might be associated with EOEE. Furthermore, the therapeutic regimen for EOEE has yet to be well elucidated. Herein, we reported the clinical and genetic features of a case with *GABRB3*-related EOEE.

Patient concerns: A 6-year-old girl developed epileptic seizures 3 days after birth. She presented with multiple seizure types including myoclonic seizures, spasms, and absence seizures. Serial electroencephalographic examinations showed variable abnormalities, and intellectual evaluation revealed significant development retardation. Conventional antiepileptic drugs were ineffective for the seizure controlling. Genetic screening identified a novel nonsense mutation (C.5G > A, p.W2X) in the *GABRB3* gene.

Diagnoses: Early onset epileptic encephalopathy.

Interventions: We changed the antiepileptic strategy to oral clonazepam (0.5mg twice daily). The patient was followed up once a week and significant declining in the attack frequency was noted 1 week later (2–3 times daily). Subsequently, the dosage was doubled (1mg twice daily), and complete cessation of seizures was achieved 20 days later.

Outcomes: Through a 9-month follow up,the girl remained seizure-free.

Lessons: This study identified a novel nonsensemutation (C.5G>A) in the exon 1 of *GABRB3* Gene, which may be associated with EOEE. To our knowledge, this is the first report to use clonazepam in the patient with *GABRB3*-related EOEE with favorable outcome. Our finding suggested that clonazepam might be a choice for patient with *GABRB3*-related EOEE. The remarkable efficacy of clonazepam in the control of seizures indicated a potential *GABRB3*- or GABA-related mechanism involved in the development of EOEE.

Abbreviations: EEG = electroencephalography, EOEE = early onset epileptic encephalopathy, GABA = gamma-aminobutyric acid, GABRB3 = gamma-aminobutyric acid A receptor, subunit beta 3, MRI = magnetic resonance imaging, NGS = next-generation sequencing.

Keywords: early onset epileptic encephalopathy, *GABRB3*, clonazepam

1. Introduction

Early onset epileptic encephalopathy (EOEE) is one of the most serious early onset epilepsies, which is characterized by progressive psychomotor impairment. The definitive etiopathogenesis of EOEE remains unclear; in literatures, the previous identified causes included intracranial structural abnormalities, neurodegeneration, metabolic diseases, and genetic defects.[1,2] With the evolution of next-generation sequencing (NGS) technology, over 100 genes, such as *SCN8A* and *CDKL5*, have been suggested to be involved in the development of EOEE.[3,4]

The gamma-aminobutyric acid A receptor, subunit beta 3 (*GABRB3*) gene, which located on chromosome 15q11.2-q12, encodes the beta-3 subunit of the gamma-aminobutyric acid (GABA) type A receptor.[2] It has been widely accepted that the GABA type A receptor plays an important role in mediating the fast inhibitory synaptic transmission in the central nervous system. Recently, *GABRB3* mutations have been identified in patients with infantile spasms and Lennox–Gastaut syndrome.[5] To date, relevant studies regarding *GABRB3*-related EOEE are sparse in the literature.[2] Furthermore, the therapeutic regimen for EOEE has yet to be well elucidated.

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Herein, we reported a case with EOEE who was effectively treated with clonazepam, and genetic screening identified a novel nonsense mutation (C.5G>A, p.W2X) in the *GABRB3* gene.

2. Case report

A 6-year-old girl, who was delivered vaginally following a 42-week gestation, developed a transient epileptic seizure 3 days after birth, which was manifested by loss of consciousness, eyes on the turn, facial cyanosis, and urinary incontinence. There was no convulsion in extremities. The seizure lasted for approximately 1 minute and resolved spontaneously. In the following months, she experienced similar attacks 2 to 3 times daily, and all these seizures occurred during daytime. Sodium valproate syrup was prescribed with a dosage of 15 mg/kg/day, whereas it provided no benefits. Intellectual evaluation showed significant development retardation.

At 2 years of age, the seizures manifested as eye deviation, left-limb convulsion and urinary incontinence. The seizure lasted for approximately 2 to 3 minutes. The attack frequency changed into 2 or 3 times every week. Brain magnetic resonance imaging (MRI) was normal. Interictal electroencephalography (EEG) revealed multifocal medium- or high-amplitude spike-slow discharges originating from bilateral frontal, parietal, temporal, and occipital lobes (predominantly from the left-sided hemisphere) (Fig. 1A).

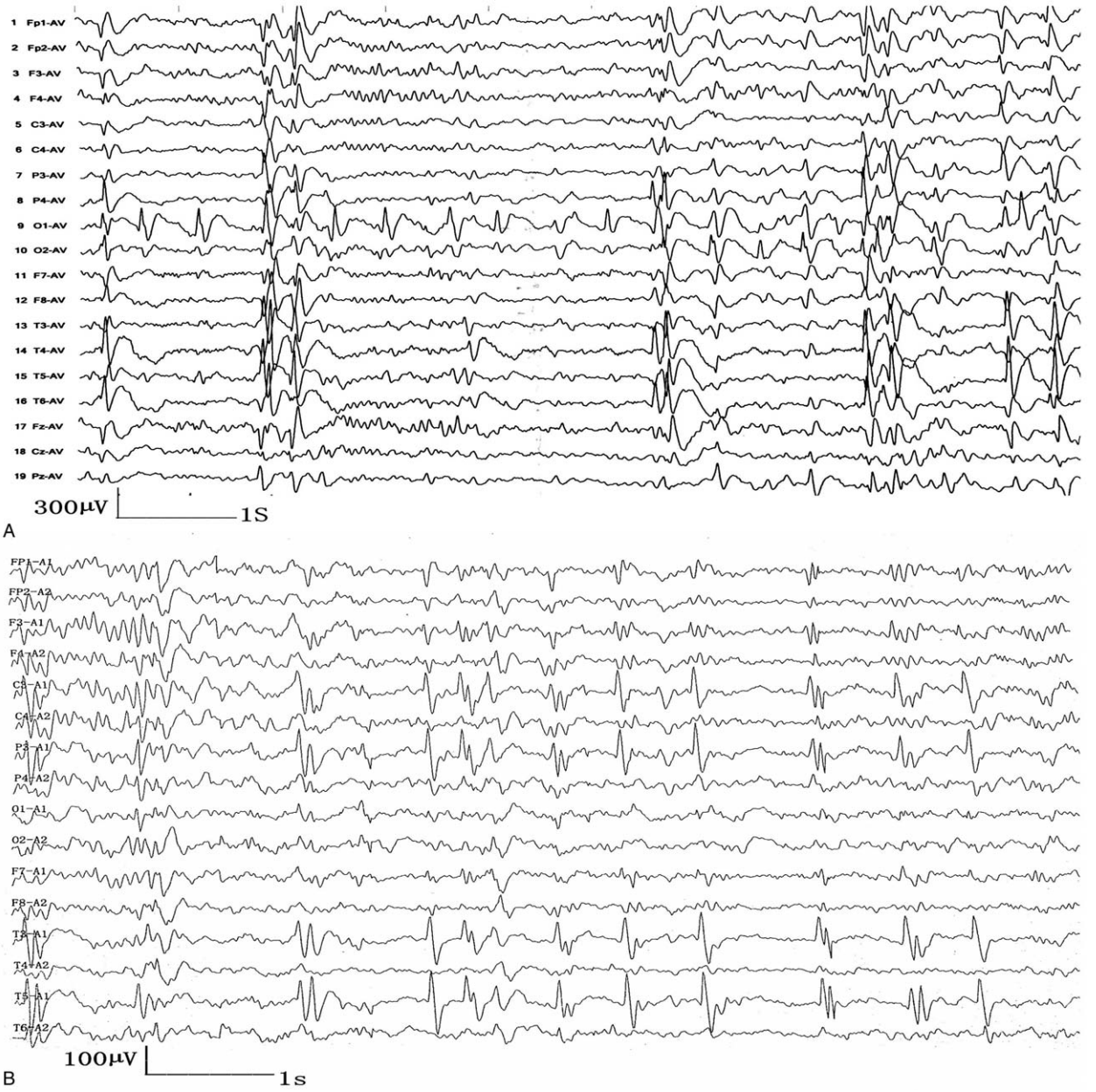

Figure 1. Electroencephalography of the patient with EOEE. (A) At 2 years of age, interictal electroencephalography revealed multifocal medium- or high-amplitude spike-slow discharges originating from bilateral frontal, parietal, temporal, and occipital lobes (predominantly from the left-sided hemisphere). (B) At 4 years of age, interictal electroencephalography showed clusters of spike-slow discharges originating from the left medial-posterior temporal lobe and the left parietal lobe. EOEE = early onset epileptic encephalopathy.

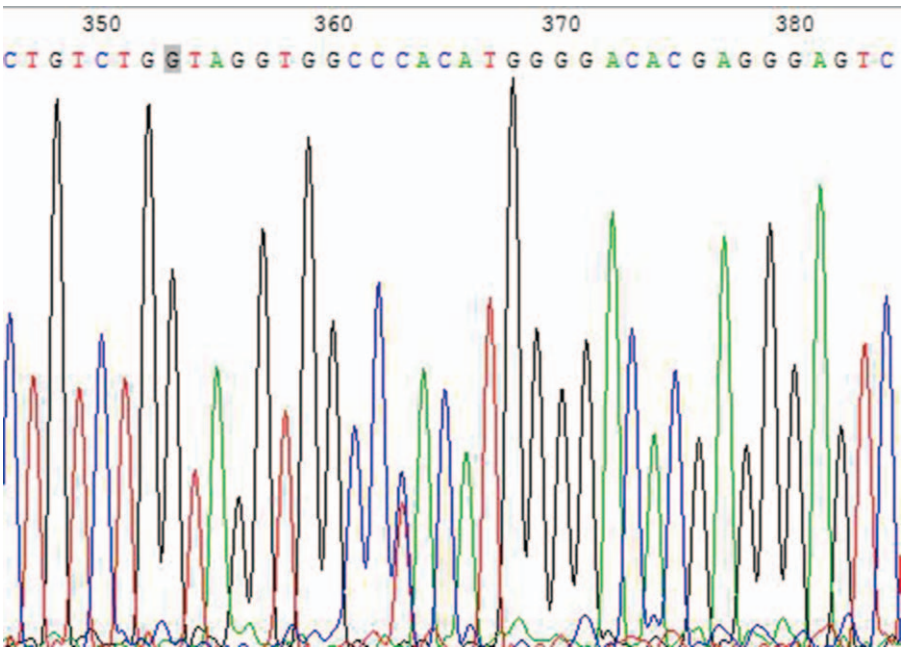

Figure 2. Bidirectional sequencing of the patient. A single heterozygous nonsense mutation (C.5G>A, p.W2X) was identified.

Oral sodium valproate was administrated with a dosage of 30 mg/kg/day, but failed to control the seizures.

At 4 years of age, the seizures manifested as sudden loss of consciousness and falling over, which lasted for half a minute with a frequency of 1 to 2 times daily. A combination regimen consisting of oral sodium valproate (30 mg/kg/day) and levetiracetam (10 mg/kg/day) was used, whereas no cessation of seizures was achieved.

Half a year prior to this admission, she presented with seizures of new type. The manifestations included nodding and shrugging for several seconds and eyes staring for 1 minute. The attack frequency increased significantly (7–8 times daily), with a peak in the morning. Her past and family medical histories were unremarkable. The physical examination was normal. EEG showed clusters of spike-slow discharges originating from the left medial-posterior temporal lobe and the left parietal lobe (Fig. 1B). Multiple antiepileptic drugs were adopted, including oral sodium valproate (30 mg/kg/day), levetiracetam (10 mg/kg/day), and topiramate (5 mg/kg/day). This strategy was still not able to control the seizures. A ketogenic diet was furtherly scheduled, but provided no efficacy. We changed the antiepileptic strategy to oral clonazepam (0.5 mg twice daily). The patient was followed up once a week and significant declining in the attack frequency was noted 1 week later (2–3 times daily). Subsequently, the dosage was doubled (1 mg twice daily), and complete cessation of seizures was achieved 20 days later. Through a 9-month follow-up, the girl remained seizure-free.

3. Genetic analysis methods

Peripheral blood samples from the patient and her parents were collected. Following a DNA extraction, genetic screening was performed using a multiple gene panel covering 524 epilepsy-related genes. A custom SureSelect library was created using the Agilent's SureDesign tool. Libraries were captured using Agilent Sureselect target enrichment system Kits (Agilent Technologies, Santa Clara, CA) and sequenced in-house on an Illumina Hiseq 2500. About 99.3% of the target regions were covered by at least 20-fold average sequencing depth. Subsequently, Sanger sequencing of both proband and parents was established to confirm the findings.

4. Genetic analysis results

A single heterozygous nonsense mutation (C.5G>A, p.W2X) was identified in the exon 1 of *GABRB3* gene (Fig. 2). Sanger sequencing showed the mutation was present in her mother and absent in her father, indicating a maternal origin. This mutation has not yet been documented in current genetic databases including ExAC (http://exac.broadinstitute.org), 1000 Genomes (http://browser.1000genomes.org), and Exome Variant Server (http://evs.gs.washington.edu/EVS). The exon 1 of *GABRB3* gene participates in the encoding of the beta-3 subunit (subtype 4) of the GABA type A receptor (NCBI accession number: NM_001191321).

5. Discussion

GABRB3 has been recently identified as a potential pathogenetic gene associated with the development of EOEE. Several cases with *GABRB3*-related EOEE have been reported in literatures.[2,6,7] We reviewed their clinical manifestations and found some common characteristics: (1) early age (<10 months) of epilepsy onset; (2) variable seizure types including myoclonus, atonic seizures, spasms, and focal seizures; (3) concomitant neuropsychological disorders, such as neurodevelopmental retardation, attention deficits, hyperactivity, and hypophrenia; (4) resistance to antiepileptic drugs; and (5) variable EEG abnormalities.[2,6,7] In the current case, the initial onset occurred just 3 days after birth, and the patients presented with multiple seizure types including myoclonic seizures, spasms, and absence seizures; serial EEGs were abnormal, and intellectual evaluation showed significant development retardation; conventional antiepileptic drugs including sodium valproate, levetiracetam, and

topiramate were all ineffective for the seizure controlling. All these features were consistent with those reported in literatures. Multiple antiepileptic drugs failed to control seizures, so clonazepam was prescribed finally, and complete cessation of seizures was achieved. To our knowledge, this is the first attempt to use this agent in the patient with *GABRB3*-related EOEE.

Genetic analysis in the present study identified a novel nonsense mutation (C.5G>A, p.W2X) in the exon 1 of *GABRB3* Gene. Exon 1 and exon 1A of *GABRB3* gene, along with exon 2–9, can generate alternative mRNA transcripts, from which 2 alternative signal peptide sequences are derived. These sequences encode 2 mature polypeptides, each of which exhibits a distinct signal peptide sequence with slight variations in N-terminal residues.[8] The mutation may lead to the structural modification of the gamma-aminobutyric acid A receptor, subunit beta 3. Furthermore, several genetic epilepsies can appear because of GABAergic disinhibition.[9] Previous studies regarding pediatric absence epilepsy have observed 3 missense mutations (c.31C>T; p.Pro11Ser), (c.44C>T; p.Ser15Phe), and (c.94G>A; p. Gly32Arg); all these were located in the exon 1A of *GABRB3* gene, and they were predicted to cause hyperglycosylation of beta 3 subunit protein in GABA type A receptor and reduction of GABA-evoked currents, thereby resulting in absence seizures.[8] In the current study, the mutation (C.5G>A, p.W2X) was found in the exon 1 which participates in the encoding of the beta-3 subunit (subtype 4) of the GABA type A receptor. We speculated this mutation may alter the structure and cause dysfunction of GABA type A receptors and thus result in EOEE. Our Sanger sequencing showed the mutation has a maternal origin, indicating a potential inheritance of EOEE. The nonsense mutation was inherited from the patient's unaffected mother, and similar cases were reported in literature.[9] The phenomenon casts some doubts on the role of the variant in the etiology of the patient' disease. It may be interpreted that by the loss of 1 allele in the patient, either it is not associated with the disease or does not show full penetrance.[9] However, due to the rarity of *GABRB3*-related EOEE, the definitive phenotype–genotype correlation between *GABRB3* mutation and EOEE is yet to be delineated, and much further studies including functional verifications are needed.

Moreover, we used clonazepam—a benzodiazepine derivative that binds to GABA type A receptors and increases the inhibitory effect of the neurotransmitter GABA[10] —to treat the patient and achieved a favorable control, which also supported the *GABRB3*-related mechanism involved in the development of EOEE. Clonazepam has been widely used in the treatment of various epileptic syndromes; nevertheless, to our knowledge, this is the first attempt to use this agent in the patient with *GABRB3*-related EOEE.

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