Received: 16 March 2023 | Accepted: 12 July 2023

DOI: 10.1002/epd2.20132

Epileptic Disorders

CLINICAL COMMENTARY

GABRB1-related early onset developmental and epileptic encephalopathy: Clinical trajectory and novel de novo mutation

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Funding information
Italian Ministry of Health

Abstract

Developmental and epileptic encephalopathy 45 (DEE45) is a neurogenetic disorder caused by heterozygous pathogenic variants of *GABRB1*, encoding the beta1 subunit of the GABA type A receptor. Only three infants with DEE45 have been reported so far, and a detailed description of the disease history of these patients is still lacking. We describe the clinical and genetic findings of a 21-year-old woman with DEE45 carrying a novel de novo *GABRB1* mutation (c.841A>G, p.T281A). The 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 antiseizure medications. Epileptic spasms 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 toward a Lennox–Gastaut Syndrome. Atypical absence status and clusters of tonic seizures occurred, often triggered by respiratory infections. The main strengths of this work are the identification of a novel pathogenic *GABRB1* variant localized in the same transmembrane domain of a previously described mutation and the detailed description of the clinical trajectory of GABRB1-related encephalopathy along 21 years of disease history.

KEYWORDS

electroencephalography, epilepsy of infancy with migrating focal seizures, epileptic spasms, GABRB1, Lennox–Gastaut syndrome

1 | INTRODUCTION

Developmental and epileptic encephalopathy 45 (DEE45) is a recently described neurogenetic disease associated with *GABRB1* pathogenic variants (OMIM #617153). Three patients with *GABRB1*-related DEE have been reported so far.[1–3] The gene *GABRB1* plays a fundamental role in central neurotransmission since it encodes the

subunit beta1 of the GABA type A receptor, a heteropentameric ligand-gated ion channel for GABA, the main inhibitory neurotransmitter in the mammalian brain.[4] GABA type A receptor is a key pharmacological target since it is the site of action of barbiturates, benzodiazepines, and ethanol.[4] Here, we report the clinical and genetic findings of a fourth patient with *GABRB1*-related DEE.

Key points

- Developmental and epileptic encephalopathy 45 (DEE45) is a neurogenetic disorder caused by heterozygous pathogenic variants of GABRB1, encoding the beta1 subunit of the GABA type A receptor.
- We describe the fourth case of DEE45:a 21- year-old woman with DEE45 carrying a novel de novo GABRB1 mutation (c.841A⟩G, p.T281A). Remarkably, the previously reported paralog GABRB3 variant p.T281A displayed a gain-of-function effect and was associated with a similar severe phenotype.
- The patient presented at birth with hypotonia and epilepsy of infancy with migrating focal seizures refractory to antiseizure medications. Acquired microcephaly, profound mental retardation and tetraparesis appeared in the first years of life.
- Epileptic spasms partially responsive to steroid therapy appeared in the second year of life.
- During childhood and adolescence, the epileptic phenotype evolved toward a Lennox-Gastaut Syndrome, in whom a partial efficacy of lamotrigine, topiramate and clonazepam was observed.

2 | METHODS

All the available clinical data and instrumental investigations performed along the patient history, including electroencephalograms (EEG) and brain magnetic resonance imaging (MRI) studies, have been collected and evaluated by an expert pediatric and adult neurologist and neurophysiologist (RD and LB). The patient was enrolled in the EPIEXOME Research Project, a study approved by the Ethics Committee of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy). A venous blood sample was collected, and genomic DNA was extracted with standard procedures. A trio-based whole-exome sequencing (WES) was performed with the SeqCap EZ/Vchrom target enrichment kit (NimbleGen) on NextSeq500 (Illumina). Reads alignment and variant calling/annotation were performed using bwa mem and GATK tools. The candidate variant was validated via Sanger sequencing in the affected subject and her parents. Written informed consent for publication of clinical details and images was obtained from the patient's parents (legal guardians). The data that support the findings of this study are available from the corresponding author upon reasonable request.

3 | RESULTS

3.1 | Clinical history

The patient is a 21-year-old female with severe neonatal-onset developmental and epileptic encephalopathy. Her clinical picture is characterized by acquired microcephaly, profound mental retardation, tetraparesis, and refractory epilepsy.

The patient was born to healthy non-consanguineous parents after an unremarkable pregnancy. At birth, weight (3040 g) and length (52 cm) were normal as well as head circumference (34 cm, 54th centile). Epilepsy started at 12 h of life with apneic episodes. At onset, seizures were characterized by apnea, behavioral arrest, tonic, and, less frequently, clonic manifestations. She had around 5–15 seizures per day. Neurologic examination showed absent visual tracking, hypotonia, and poor spontaneous movements. Interictal EEG was characterized by mixed frequency activity (delta–theta–beta) with poor awake–sleep differentiation and right posterior spikes. Ictal EEG showed rhythmic theta activity occasionally preceded by low-amplitude beta activity with onset in the right temporal region with a duration of 15–30 s. The EEG pattern showed multifocal seizures, initially with a more frequent onset in the posterior right region. In the first months of life, the features evolved toward the epileptic syndrome nowadays known as epilepsy of infancy with migrating focal seizures (EIFMS). In the first months of life, many drugs such as clobazam, valproic acid, phenytoin, phenobarbital, carbamazepine, and pyridoxine were consecutively tried without significant efficacy. Brain MRI was normal, such as visual and auditory evoked potentials, electroretinography, and electromyography. Extensive metabolic tests on blood, cerebrospinal fluid, and urine did not reveal abnormalities. Karyotype and comparative genomic hybridization array (CGH-array) analysis were normal. Ceroid lipofuscinoses were excluded with analyses on skin-derived fibroblasts. Fluorescent in situ hybridization (FISH) analysis for Prader–Willi syndrome was negative.

After the first year of life, EEG evolved toward a disorganized background with generalized slow activity mixed with multifocal and generalized sharp waves and spike–wave discharges. At the end of the first year of life, her head circumference growth was gradually slowing (43.9 cm, 31st centile). Between the first and second year of life, seizures increased in frequency and duration, up to 2–3 min, and in addition to the previous seizure types, epileptic spasms (ES) and irregular myoclonus associated with polyspike activity were recorded at EEG. Synthetic adrenocorticotropic hormone (ACTH), tetracosactide, was administered with spasm reduction, EEG spike frequency reduction, and sleep–wake cycle improvement. Therapy was then switched to lamotrigine and clonazepam. At that time, brain MRI was repeated, showing a mild white matter hyperintensity. From the third year of life, she had a spontaneous reduction of focal seizures (2–3 seizures per week), though intellectual and motor functions were severely impaired with head growth stagnation (head circumference of 47.2 cm at almost 4 years of age, sixth centile).

At the age of 6 years, the patient presented severe clusters of tonic–clonic seizures (approximately 1 or 2 days per month) requiring the oral administration of clonazepam. Levetiracetam was then added. The clinical picture was complicated by severe gastroesophageal reflux, dysphagia with recurrent inhalation, restrictive pulmonary syndrome, and obstructive sleep apnea syndrome. Therefore, a percutaneous endoscopic gastrostomy (PEG) tube was placed and assisted ventilation during night sleep was started. She presented tonic seizures also during sleep. At that time, brain MRI showed only a mild enlargement of the third and lateral ventricles and a mild thinning of corpus callosum, with no parenchymal altered signal abnormalities (Figure 1A).

In the late childhood period, the patient was repeatedly admitted to the hospital for respiratory infections and severe cluster of seizures. Background EEG showed a diffuse theta–delta slow activity with intermittent polyspike-and-waves, more prominent on frontal regions. Episodes of atypical absence status epilepticus with prolonged 2 Hz spike-and-wave discharges (Figure 1B) and tonic seizures (Figure 1C) were documented. The electroclinical phenotype at that point was clearly compatible with Lennox–Gastaut syndrome (LGS). Topiramate was added with reduction of seizures and hospitalizations.

At the last evaluation (21 years old), the patient was treated with lamotrigine, levetiracetam, and topiramate. Epilepsy manifested with weekly brief tonic seizures. The girl was severely disabled, wheelchair-bound with no head control. She was microcephalic (head circumference: 49.5 cm, less than first centile). Communication ability was absent. Feeding and hydration were totally ensured by the PEG tube. Vineland-II standard assessment, the most used tool to quantify adaptive behavior impairment, showed profound impairment levels with a score of around 20 in the three investigated domains (communication, autonomy in daily activities, and socialization).[5]

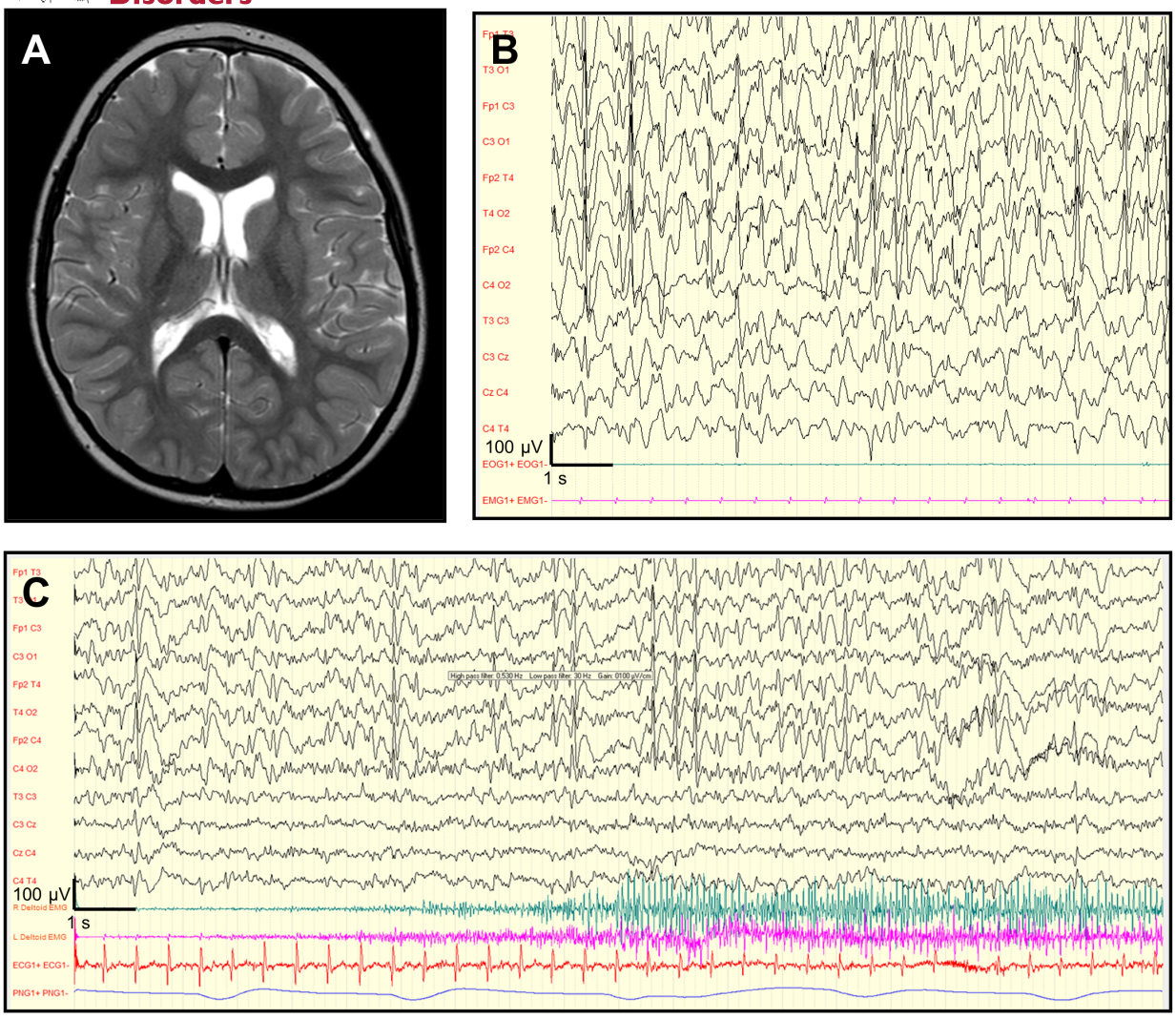
3.2 | Genetic analysis

A filtering analysis of trio WES data looking for de novo variants in the proband revealed the novel heterozygous *GABRB1* c.841A>G, p.T281A variant (NM_000812.4). De novo occurrence was confirmed by paternity testing. No other candidate variants in other genes associated with epilepsy were found in the patient. The p.T281A variant was predicted pathogenic by all in silico prediction tools tested (CADD, DANN, Polyphen2, SIFT, and REVEL) and affected a highly conserved amino acidic residue in orthologs. In silico structural analyses using the online DynaMut software predicted that the p.T281A causes an increase in molecule flexibility compared to wild-type protein.[6] For these reasons, this variant was classified as *pathogenic* following the ACMG guidelines (PS2s, PP3s, and PM2m).

4 | CONCLUSIONS

We reported the clinical trajectory of a *GABRB1* DEE patient from the neonatal to the adult age. The three previous published infants with the same disease are summarized in Table 1. *GABRB1* was first associated with an epileptic phenotype in 2013, when the Epi4K Consortium and Epilepsy Phenome/Genome Project identified a single de novo heterozygous missense variant (c.737T>C, p.F246S) studying through WES a large cohort of 264 DEE patients with infantile spasms and LGS phenotypes. This patient had a DEE with focal seizure onset at 12 months and developmental regression from the age of 35 months with ES, atypical absence, atonic, myoclonic seizures. EEG showed hypsarrhythmia and, at 4 years of age, 2 Hz spike wave discharges. At the last follow-up (4.5 years old), he had global developmental delay, hypotonia, ataxia, severe dysphagia (gastrostomy tube), cortical visual impairment, and thin corpus callosum at brain MRI.[1] In 2016, Janve et al. published functional studies on this variant. Inserting the mutant channels in HEK293 cells, they showed that the mutation altered the kinetic properties of the channel, resulting in the net loss of GABAergic inhibition.[7]

A second boy with drug-resistant DEE45 was reported by Lien et al. in 2016: A de novo heterozygous missense mutation (c.860C>T, p.T287I) was found through WES. Ketogenic diet had some positive effects.[2]



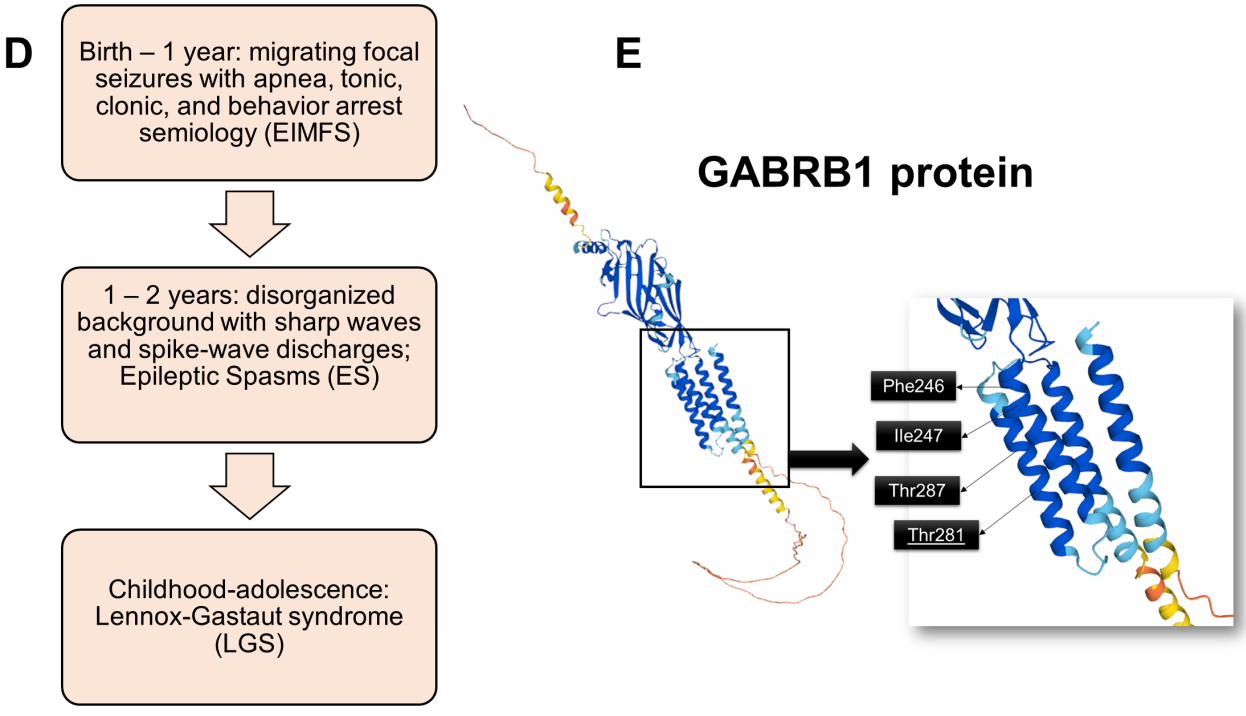


FIGURE 1 Clinical course and genetic features of the patient: (A) Axial T2-weighted brain MRI of the patient performed at the age of 6 years, showing a mild enlargement of the third and lateral ventricles; (B) EEG performed at the age of 14 showing nonconvulsive atypical absence status epilepticus; (C) EEG polygraphy performed at the age of 17 displaying the ictal EEG features of a tonic seizure; (D) Graphical representation of the clinical course of the patient; (E) 3D view of the GABRB1 protein (AlphaFold) indicating the positions of the identified pathogenic variants.

TABLE 1 Literature review of published DEE45 clinical cases.

Patient	1[1]	2[2]	3[3]	4 (this case)
Mutation	p.F246S	p.T287I	p.I247T	p.T281A
Inheritance	AD (de novo)	AD (de novo)	AD (de novo)	AD (de novo)
Phenotype	IS	DEE	EIFMS	EIMFS > IS> LGS
Age of onset	12 months	3 months	4 months	First day of life
Initial seizure types	Focal seizures	NA	Focal	Migrating focal seizures (MFS) with autonomic or tonic semiology
Additional seizure types	ES at 35 months, atypical absence, atonic, and myoclonic seizures	NA	Focal, tonic	ES at 1.5 years; atypical absences, tonic and tonic–clonic seizures during childhood–adolescence
EEG	Hyps at 35 months, 2 Hz spike wave at 4 years	NA	Disorganized background and multifocal discharges at interictal EEG	Ictal rhythmic beta–theta–delta activity during MFS, EEG background poor organization at onset; disorganized background with sharp waves and spike–wave discharges at 1–2 years; recurrent spike–wave discharges during childhood–adolescence
Brain MRI	Thin corpus callosum, otherwise normal	Normal	Progressive white matter atrophy (corpus callosum, frontal and temporal lobes)	Normal in the first months of life; white matter hyperintensity, at 1.5 years, mild enlargement of the third and lateral ventricles and mild thinning of corpus callosum at 6 years
NE and development before seizure onset	Gross, fine motor delay, hypotonus, cortical visual impairment, and regression after seizure onset	Hypotonia	Hypotonia dysmorphism, autistic features	Hypotonia, no visual track
NE and development at last evaluation	At 4.5 years: global delay, low muscular tone, ataxia, cortical visual impairment, and gastrostomy tube	At 2.7 years: profound developmental delay	At 2 years: profound developmental delay (seizure free)	At 21 years: Profound intellectual and motor disability, absent communication, severe visual impairment, hypotonia, tetraparesis, and microcephaly
Therapies	NA	Drug-resistant epilepsy, positive effect for ketogenic diet	NA	Poor efficacy for clobazam, valproic acid, phenytoin, phenobarbital, carbamazepine, and pyridoxine; partial efficacy of tetracosactide, lamotrigine, topiramate, and clonazepam

Abbreviations: AD, autosomal dominant; EIFMS, epilepsy of infancy with migrating focal seizures; ES, epileptic spasms; Hyps, hypsarrhythmia; IS, infantile spasms; MFS, migrating focal seizures; NA, not available; NE, neurologic examination.

A third patient was reported in 2019 by Burgess et al.: A 2-year-old girl, born to unrelated parents of Italian and Moroccan origin, affected by severe developmental delay and EIMFS, carrying a de novo heterozygous missense mutation (c.740T>C, p.I247T).[3]

This case displayed in the adult age a severe neurologic phenotype characterized by profound mental retardation, acquired microcephaly, tetraparesis, and refractory epilepsy. Her epileptic phenotype evolved over time, turning from EIFMS in first months of life with addition of ES in

the late infancy to LGS during late childhood–adolescence (Figure 1D).

The identified novel variant (NM_000812.4: c.841A>G, p.T281A) is localized in the proximity of the previously described *GABRB1* pathogenic variant p.T287I (Lien et al. in 2016), affecting the same alpha-helical transmembrane domain. Interestingly, all the reported *GABRB1* pathogenic variants affect transmembrane domains of the GABRB1 protein, suggesting a similar molecular pathogenic mechanism causing DEE45 (Figure 1E).[1,2,7]

Several missense mutations in the *GABRB3* gene, a paralogous gene of *GABRB1*, encoding the subunit beta3 of the GABA type A receptor, have been functionally characterized by Absalom et al.[8] Remarkably, the paralog *GABRB3* variant p.T281A, reported as the genetic cause of neonatal-onset EIFMS in monozygotic twins,[9] displayed a gain-of-function effect, which appears to be associated with younger age of seizure onset, higher risk of severe intellectual disability, and lower likelihood of seizure control in comparison to loss-of-function variants. This finding suggests that the current case, also presenting a severe drug-resistant developmental and epileptic encephalopathy, may be due to a gain-of-function effect, with a possible impact on therapeutic approach.

The main strengths of this work, reporting the fourth case of *GABRB1*-related DEE, are the description of the clinical and instrumental trajectory of the patient along the 21 years of her life and the identification of a novel de novo pathogenic *GABRB1* variant (c.841A>G, p.T281A). *GABRB1* should be considered in patients with early infantile focal seizures and EIMFS phenotype, as they seem the most peculiar presentation before evolution into other seizure types and neurologic regression. Given the few cases described, a large international multicenter collection of patients could be the next step to better describe the phenotypic spectrum.

FUNDING INFORMATION

This study was supported by the Italian Ministry of Health (Ricerca Corrente).

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Monfrini E, Borellini L, Zirone E, Yahya V, Mauri E, Molisso MT, et al. *GABRB1*-related early onset developmental and epileptic encephalopathy: Clinical trajectory and novel de novo mutation. Epileptic Disord. 2023;25:867–873. https://doi.org/10.1002/epd2.20132

Test yourself

1. *GABRB1* pathogenic variants associated with developmental and epileptic encephalopathy 45 (DEE45) are localized in the following protein domain of the beta1 subunit of the GABA type A receptor:
   A. Extracellular domain
   B. Intracellular domain
   C. Transmembrane alpha-helical domain
   D. None of the above
2. Which of the following epileptic syndromes may be associated with *GABRB1*-related developmental and epileptic encephalopathy?
   A. Epileptic spasms
   B. Lennox–Gastaut syndrome
   C. Epilepsy of infancy with migrating focal seizures
   D. All of the above
3. Concerning the epileptic manifestations in the long-term history of *GABRB1*-related developmental and epileptic encephalopathy:
   A. The epileptic phenotype may evolve over time
   B. In all patients, the epileptic phenotype is static over time
   C. All patients present Lennox–Gastaut syndrome
   D. All patients have infantile spasms

Answers may be found in the supporting information.