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RESEARCH ARTICLE

Molecular and clinical descriptions of patients with $GABA_A$ receptor gene variants (*GABRA1, GABRB2, GABRB3, GABRG2*): A cohort study, review of literature, and genotype–phenotype correlation

Pierre-Yves Maillard[1] | Sarah Baer[2,3] | Élise Schaefer[1] | Béatrice Desnous[4] | Nathalie Villeneuve[4] | Anne Lépine[4] | Alexandre Fabre[5,6] | Caroline Lacoste[7] | Salima El Chehadeh[1,3] | Amélie Piton[3,8] | Louise Frances Porter[9] | Caroline Perriard[2] | Marie-Thérèse Abi Wardé[2] | Marie-Aude Spitz[2] | Vincent Laugel[2] | Gaëtan Lesca[10] | Audrey Putoux[10] | Dorothée Ville[11] | Cyril Mignot[12,13] | Delphine Héron[12,13] | Rima Nabbout[14] | Giulia Barcia[15] | Marlène Rio[15] | Agathe Roubertie[16] | Pierre Meyer[16] | Véronique Paquis-Flucklinger[17] | Olivier Patat[18] | Jérémie Lefranc[19] | Marion Gerard[20] | Epigen Consortium | Julietta de Bellescize[21] | Laurent Villard[5,22] | Anne De Saint Martin[2,3] | Mathieu Milh[4,22]

[1]Department of Medical Genetics, IGMA, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[2]Department of Neuropediatrics, ERN EpiCare, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[3]Institute for Genetics and Molecular and Cellular Biology (IGBMC), University of Strasbourg, CNRS UMR7104, INSERM U1258, Illkirch, France

[4]Department of Pediatric Neurology, AP-HM, La Timone Children's Hospital, Marseille, France

[5]Pediatric Multidisciplinary Unit, AP-HM, Timone Enfant, Marseille, France

[6]Aix-Marseille University, INSERM, GMGF, Marseille, France

[7]Department of Medical Genetics, La Timone Children's Hospital, Marseille, France

[8]Laboratory of Genetic Diagnosis, Institut de Génétique Médicale d'Alsace, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[9]Department of Medical Genetics, Institut de Génétique Médicale d'Alsace, Centre de Référence pour les Affections Rares en Génétique Ophtalmologique (CARGO), Strasbourg, France

[10]Department of Genetics, Hospices Civils de Lyon, Bron, France

[11]Pediatric Neurology Department and Reference Center of Rare Epilepsies, Mother Child Women's Hospital, Lyon University Hospital, Lyon, France

[12]Department of Genetics, Groupe Hospitalier Pitié-Salpêtrière and Hôpital Armand Trousseau, APHP-Sorbonne Université, Paris, France

[13]Centre de Référence Déficiences Intellectuelles de Causes Rares, Paris, France

[14]Department of Pediatric Neurology, Reference Centre for Rare Epilepsies, Necker Enfants Malades University Hospital, APHP, Université de Paris, Paris, France

[15]Department of Medical Genetics, Necker-Enfants Malades Hospital, Université de Paris, Paris, France

[16]Pediatric Neurology Department, INM, INSERM, CHU Montpellier, University of Montpellier, Montpellier, France

[17]Department of Medical Genetics of Nice University Hospital, Nice, France

[18]Department of Medical Genetics, CHU Toulouse Purpan, Toulouse, France

Pierre-Yves Maillard and Sarah Baer contributed equally.

[19]Pediatric Department, CHU de Brest, Brest, France

[20]Department of Medical Genetics, Centre Hospitalier Universitaire de Caen, Caen, France

[21]Paediatric Clinical Epileptology and Functional Neurology Department, Reference Center of Rare Epilepsies, Member of the ERN EpiCARE, University Hospitals of Lyon (HCL), Lyon, France

[22]Faculté de Médecine Timone, Aix Marseille Univ, INSERM, MMG, U1251, ERN Epicare, Marseille, France

Correspondence
Mathieu Milh, Pediatric Neurology Department, Timone Children Hospital, 264 Rue Saint Pierre, 13005 Marseille, France.
Email: mathieu.milh@ap-hm.fr; mathieu.milh@gmail.com

Present address
Pierre-Yves Maillard, Institut Jérome Lejeune, Paris, France

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Abstract

Objective: γ-Aminobutyric acid $(GABA)_A$-receptor subunit variants have recently been associated with neurodevelopmental disorders and/or epilepsy. The phenotype linked with each gene is becoming better known. Because of the common molecular structure and physiological role of these phenotypes, it seemed interesting to describe a putative phenotype associated with $GABA_A$-receptor–related disorders as a whole and seek possible genotype–phenotype correlations.

Methods: We collected clinical, electrophysiological, therapeutic, and molecular data from patients with $GABA_A$-receptor subunit variants *(GABRA1, GABRB2, GABRB3,* and *GABRG2)* through a national French collaboration using the EPIGENE network and compared these data to the one already described in the literature.

Results: We gathered the reported patients in three epileptic phenotypes: 15 patients with fever-related epilepsy (40%), 11 with early developmental epileptic encephalopathy (30%), 10 with generalized epilepsy spectrum (27%), and 1 patient without seizures (3%). We did not find a specific phenotype for any gene, but we showed that the location of variants on the transmembrane (TM) segment was associated with a more severe phenotype, irrespective of the $GABA_A$-receptor subunit gene, whereas N-terminal variants seemed to be related to milder phenotypes.

Significance: $GABA_A$-receptor subunit variants are associated with highly variable phenotypes despite their molecular and physiological proximity. None of the genes described here was associated with a specific phenotype. On the other hand, it appears that the location of the variant on the protein may be a marker of severity. Variant location may have important weight in the development of targeted therapeutics.

KEYWORDS

channelopathy, developmental and epileptic encephalopathy, GABA A receptor, genetic generalized epilepsy

1 | INTRODUCTION

GABA (γ-aminobutyric acid) is the most abundant inhibitory neurotransmitter,[1] and its receptor is an important pharmacological target of many antiseizure medications.[2,3] Three major receptor classes are activated by GABA: the abundant GABA type A receptors, which are ligand-dependent, postsynaptic anion channels that exert a rapid inhibitory action when activated[4]; the metabotropic G protein–coupled GABA type B presynaptic receptors[5]; and the less widespread GABA type C receptors.[6] $GABA_A$-receptor subunits are combined as heteropentamers from various combinations of proteins—most frequently two α,

Key points

- γ-Aminobutyric acid $(GABA)_A$–receptor subunit variants lead to a fever-related epilepsy, a generalized epilepsy, or a developmental and epileptic encephalopathy
- Phenotype was not associated with a given gene, but with the location of variants within the protein
- Variants of the transmembrane domain are significantly more severe than other variants in our cohort and in 402 published cases.

two β, and one γ subunit.[7] All $GABA_A$-receptor subunits exhibit a similar structure, with four transmembrane domains (TM1 to TM4) and a long extracellular N-terminal domain (NT). The ion channel pore is formed by the second transmembrane domain (TM2) of each subunit. These receptors include several binding sites for ligands (agonist, antagonist, or benzodiazepine [BZD][8,9]).

$GABA_A$-receptor subunit heterozygous variants have recently been identified as an important cause of childhood epilepsy with or without intellectual disability (ID).[10] $GABA_A$-receptor subunit variants were first identified in *GABRG2* and *GABRA1* using classical linkage analysis, and then candidate gene sequencing in large families with autosomal dominant genetic generalized epilepsy.[4,11] With the availability of next-generation sequencing, many different epileptic phenotypes have been linked to $GABA_A$-receptor subunit variants with or without neurodevelopmental disorder,[2] these genes being one of the most frequently implicated in epilepsy phenotypes.[12] Given the structural and functional proximity of $GABA_A$-receptor subunits, it seemed relevant to leave the gene-by-gene description and consider as a coherent whole the disorders associated with $GABA_A$-receptor subunit mutations. This approach has been proposed recently and guided us for this study at the clinical, electrophysiological, and developmental levels.[13–16]

Here, we report a series of 37 patients with pathogenic variants in one of the $GABA_A$-receptor subunits—*GABRA1*, *GABRB2*, *GABRB3*, and *GABRG2*—and we analyzed 402 cases reported in the literature, identifying the clinical, electrophysiological, and molecular characteristics, with an emphasis on genotype–phenotype correlations and protein domain phenotype correlations to highlight the link between variant localization and neurodevelopmental impairment severity.

2 | MATERIAL AND METHODS

We collected clinical, electrophysiological, therapeutic, and molecular data from patients affected with the most frequent $GABA_A$-receptor subunit variants (*GABRA1*, *GABRB2*, *GABRB3*, and *GABRG2*) through a national French collaboration using the EPIGENE network. We used a standardized survey completed by the clinician following these patients. Seizures were classified according to the International League Against Epilepsy (ILAE) classification.[17] This study has been declared to the health data access portal of Assistance Publique-Hôpitaux de Marseille under the reference YNRSXY and registered under the number PADS22-23.

We gathered epileptic phenotypes in three spectrums using the ILAE diagnosis criteria:

1. Epilepsy associated with fever sensibility: Genetic epilepsy with febrile seizures plus (GEFS+), Dravet syndrome (DS) spectrum, and epilepsies in which fever sensitivity is a preeminent sign.
2. Early developmental epileptic encephalopathy (EDEE) comprising epilepsy of infancy with migrating focal seizures (EIMFS), early infantile epileptic encephalopathy (EIEE), and early-onset epilepsies that did not fit with any epileptic syndrome.
3. Genetic generalized epilepsy: epilepsy with myoclonic-atonic seizure (MAE), juvenile myoclonic epilepsy (JME), atypical absences, and unclassified generalized epilepsy with normal background activity and generalized paroxysmal activities. MAE epilepsy was retained if seizures with myoclonic-atonic falls were present, whether or not associated with other types of seizures (atypical absences, generalized tonic-clonic seizures, myoclonia, and so on). Electroencephalography (EEG) could show focal or generalized anomalies.

Cognitive development assessment was based predominantly on psychomotor development assessed by the referring physicians, with occasional formal neuropsychological testing. Informed consent for study inclusion was obtained from patients and parents or their legal guardians, in compliance with the Declaration of Helsinki.

At the molecular level, we considered variants as pathogenic based on a combination of the following criteria as suggested by Richards et al. (2015)[18]: (1) the presence of nonsense, missense, nonsynonymous, frameshift, and splicing modifier variants; (2) the absence of variants from human polymorphism databases (such as gnomAD[19]); (3) predicted as pathogenic by in silico prediction software (SIFT,[20] PolyPhen,[21] MutationTaster,[22] and UMD Predictor[23]); (4) the results of segregation analyses with the presence of a variant in the affected individual or transmission by an affected parent. All variants are heterozygous except in three siblings with a homozygous variant in a consanguineous pedigree. All variants were identified using high-throughput targeted gene-sequencing panels designed for neurodevelopmental disorders (ID and epilepsy panels) except for patient 4, for whom whole-exome sequencing was performed. All variants were confirmed by Sanger sequencing, as was familial segregation.

2.1 | Literature review

We analyzed all cases reported to date (March 2022) with a pathogenic mutation of one of the following $GABA_A$-receptor subunits: *GABRA1*, *GABRB2*, *GABRB3*, and *GABRG2* using the HGMD Pro database[24] and PubMed website. English-language articles were selected.

For each case, we noted the clinical phenotype according to the classification previously described: (1) epilepsy associated with fever sensibility, (2) early developmental epileptic encephalopathy, and (3) genetic generalized epilepsy. The cases for which the classification was not possible due to missing data or for which the epileptic phenotype did not fit with any of these three phenotypes have nevertheless been reported. We also collected the age at the first seizure and the severity of ID, when available.

2.2 | Statistical analysis

Descriptive statistics are reported as frequencies (sample size and percentages) and means for categorical and continuous variables, respectively. The groups were compared using χ^2 or Fisher exact tests as appropriate. All analyses were performed using IBM SPSS Statistics 20.0 (IBM Inc.). For all two-tailed tests used, a significance level of $p < .05$ was considered statistically significant.

3 | RESULTS

We report 34 unpublished patients with a pathogenic variant in $GABA_A$-receptor subunits (3 of them have already been reported in Johannesen et al.[25]): 6 in *GABRA1*, 5 in *GABRB2*, 16 in *GABRB3*, and 10 in *GABRG2*, representing 16 male and 21 female patients with an age at epilepsy onset from birth to 16 years. Thirty-four index cases with heterozygous variants and three sibling cases with a homozygous variant are presented. All clinical and molecular results are summarized in Table 1.

3.1 | Clinical results

3.1.1 | Developmental and electroclinical phenotypes

The analysis of the electroclinical phenotype of the patients led us to describe three groups:

3.1.1.1 | *Group 1: Fever-related epilepsy (40%—n = 15)*

This group includes six patients with a *GABRG2* variant, four with a *GABRB3* variant, three with a *GABRA1* variant, and two with a *GABRB2* variant. The epilepsy began on average at 10.9 months (range 3–26 months) with fever-related generalized tonic-clonic seizures ($n = 12$) and/or focal seizures ($n = 7$). Some patients also had other types of seizures at first evaluation: absence seizures ($n = 6$), atonic seizures ($n = 5$), myoclonic seizures ($n = 3$), and clonic seizures ($n = 2$). One patient also had an alternating hemiplegia (Patient 25, Figure 1A). Initial EEG abnormalities were generalized ($n = 4$); two patients had focal anomalies and three have multifocal anomalies. Seven of these patients had a normal EEG at first evaluation. Most of the patients from this group had a moderate intellectual disability (or ID) ($n = 7$), three with mild ID and two with severe ID; it should be noted that three patients had no ID (Figure 1B). The most effective antiseizure medications (ASMs) were valproic acid ($n = 10$), clobazam and other benzodiazepines alone or in association ($n = 6$), and lamotrigine ($n = 4$). One patient became seizure-free under a ketogenic diet. All the patients of this group had good control of their epilepsy under ASMs at the end of the follow-up. Five patients had electroclinical features of Dravet syndrome (Table 1).

3.1.1.2 | *Group 2: Early developmental epileptic encephalopathy (EDEE) (29%—n = 11)*

Six patients had a variant in *GABRB3*, including three siblings; two had a variant in *GABRB2*, two had a variant in *GABRA1*, and one had a variant in *GABRG2*. The epilepsy began before 6 months of age, with motor symptoms in all cases: clonic and tonic seizures ($n = 8$) or clonic/myoclonic seizures ($n = 4$). One patient had focal migrating clonic seizures (Patient 21), and two patients also had infantile spasms (Patients 28 and 29) (Table 1 and Figure 1A). Of interest, we found a recurrent EEG pattern in those patients; most of them (6/11) had a suppression burst on the initial EEG, 2 of 11 patients had hypsarrhythmia, 1 had generalized spike waves, and 1 had migrating polyspike waves. One patient had a normal initial EEG. Ongoing EEGs showed asymptomatic burst of slow waves, predominantly anterior (Figure 2). This abnormal activity was not symptomatic, lasted for 5 to 20 seconds, and occurred preferentially in the quiet vigil and sleep states. All the patients had a severe ID (four patients died between 2 months and 18 years) (Figure 1B). Development was abnormal before or at epilepsy onset in all cases. The most effective ASMs were phenobarbital ($n = 4$) and valproic acid ($n = 3$), but most of the patients in this group had drug-resistant epilepsy.

3.1.1.3 | *Group 3: Generalized epilepsy spectrum (27%—n = 10)*

Six patients had a *GABRB3* variant, two had a *GABRB2* variant, and two had a *GABRG2* variant (Figure 1A). Epilepsy began at a mean age of 37 months (range 2 months to 16 years) with generalized seizures, including generalized tonic–clonic seizures ($n = 7$), absences ($n = 6$), myoclonic ($n = 4$), atonic ($n = 4$), and spasms ($n = 2$). One patient had infantile spasms and then generalized tonic–clonic seizures (patient 9). In this group, the patients endured various types

TABLE 1 Clinical characteristics of *GABRA1*, *GABRB2*, *GABRB3*, and *GABRG3* patients from our national cohort

Patient (gender)	Gene	Mutation inheritance	Age at onset	Seizure types	Epileptic phenotype	Diagnosis spectrum	ID	Clinical features	First EEG	Brain MRI	Effective drugs
N-terminal domain											
1 (F)	*GABRA1*	c.274T > C p.Phe92Leu de novo	NP	GTCS	GEFS+	Fever-related epilepsy	Moderate	Limb tremor	Normal rhythm activity with localized fast rhythmic activity and GSW	Normal	LEV, LTG, PMP
2 (F)		c.335G > A p.Arg112Gln de novo	26m	Atypical absence, GTCS	GEFS+	Fever-related epilepsy	Moderate	ASD, regression	Photo S, normal rhythm activity (34m)	Ventriculomegaly, thin CC	VPA, TPM, LTG
3 (M)		c.335G > A p.Arg112Gln de novo	7m	GTCS	GEFS+	Fever-related epilepsy	No	Macrocephaly, macrosomia	Normal (2y)	Normal	VPA, TPM, CLB
4 (F)	*GABRB3*	c.229G > A p.Glu77Lys de novo	16y	GTCS, myoclonia	MJE	Generalized epilepsy spectrum	Moderate	Trunk dystonia, spasticity, cerebellar syndrome, bilateral optic atrophy	Generalized SW (16y)	Normal	LTG, ZNS
5 (M)		c.238A > G p.Met80Val de novo	8m	Focal, myoclonic	MAE	Generalized epilepsy spectrum	Moderate	Ataxia, stereotypies, ogival palate and dysmorphism	Angelman like trace (4y)	Normal	TPM, LTG, PIR
6 (M)		c.343C > T p.Gln115* *Inherited father*	36m	Atypical absences	unclassified	Generalized epilepsy spectrum	Mild	ASD, macrosomia	Regular generalized SW 3 Hz	NP	VPA, LTG
7 (F)		c.358G > A p.Asp120Asn de novo	11m	Atypical absence, GTCS and atonic	MAE	Generalized epilepsy spectrum	No	Congenital macrocephaly	Slow bilateral SW (15m)	Normal	LTG, LEV
8 (F)		c.674 T > C, p.Phe225Ser de novo	13m	Nighttime focal secondarily generalized	GEFS+	Fever-related epilepsy	Moderate	Stereotypies, strabismus, severe GOR	Normal (<2y)	Arachnoid cyst	TPM
9 (F)		c.674 T > G p.Phe225Cys de novo	2m	Spasms, GTCS	Unclassified	Generalized epilepsy spectrum	Mild	Ataxia, tremor, nystagmus, auto-aggressivity, dyspraxia, neuro visual impairment, microcephaly	Right occipital slow waves; Angelman like	Normal	LTG, CLB
10 (M)		c.695G > A p.Arg232Gln de novo	9m	GTCS, myoclonic atonic	Incomplete Dravet	Fever-related epilepsy	Moderate	ADHD	GSW (5y)	Normal	VPA, ZNS
11 (F)		c.695G > A p.Arg232Gln *Inherited (mosaicism)*	18m	Focal, GTCS	Incomplete Dravet	Fever-related epilepsy	Moderate	Behavior problems, ADHD, morbid obesity	Bifrontal SW asymptomatic	Hypersignal WM right temporal	VPA, CBZ, CLB
12 (M)			18m	Focal, GTCS, atypical absences, atonic	GEFS+	Fever-related epilepsy	Moderate	Left foot and ankle deformation, macrocephaly	Asymptomatic GSW (2y)	Normal	LVT
13 (F)	*GABRG2*	c.282_306dup, p. Pro103Argfs*6 *Inherited*	12m	FS, GTCS	GEFS+	Fever-related epilepsy	No	NP	Normal (age NP)	Normal	LTG

(Continues)

TABLE 1 (Continued)

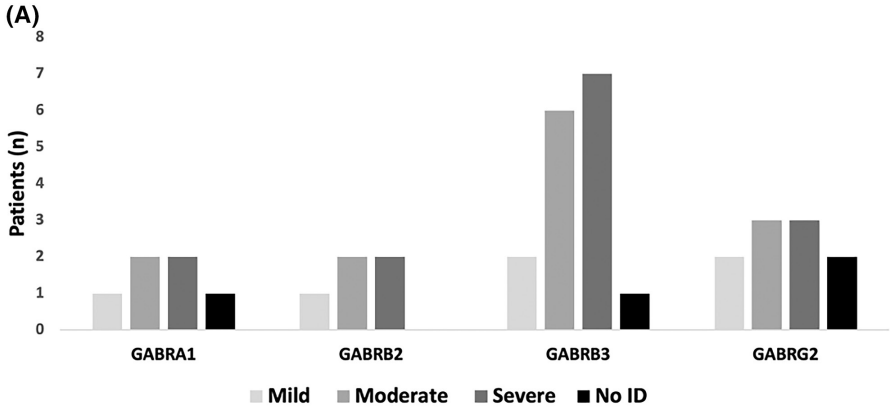
Patient (gender)	Gene	Mutation inheritance	Age at onset	Seizure types	Epileptic phenotype	Diagnosis spectrum	ID	Clinical features	First EEG	Brain MRI	Effective drugs
Transmembrane localization											
14 (M)	*GABRA1*	c.787A > G p.Met263Val de novo	1m	Myoclonic, tonic, atonic, GTCS	EIEE	EDEE	Severe	Postnatal microcephaly, hypotonia, choreoathetoid movement, nystagmus	Suppression burst (5m)	Normal	CBZ, PMP
15 (F)		c.851 T > C p.Val284Ala de novo	0.5m	Tonic, clonic	EIEE	EDEE	Severe (died at 8m)	Microcephaly, axial hypotonia, growth delay	Hypsarrhythmia (7m)	Cortical and CC atrophia	VGB
16 (M)		c.888G > T p.Leu296Phe de novo	No	No	NP	No	Mild	Drooling, bruxism, anxiety, growth delay	Normal (2y)	Normal	No
17 (M)	*GABRB2*	c.815C > G p.Ala272Gly de novo	4.5y	Night tonic clonic	GTCA	Generalized epilepsy spectrum	Moderate	Normal	Brief flashes of temporal ample bilateral spikes during sleep (4.5y)	NP	CLB, OXC
18 (M)		c.847C > A p.Leu283IleI de novo	3d	Clonic, automatism (chewing), migrating	EIEE	EDEE	Severe (died at 2m)	Axial hypotonia, peripheral hypertonia, no ocular interaction	Suppression burst	Diffuse hypersignal of the WM	Pharmacoresistant
19 (F)	*GABRB3*	c.851 T > C p.Leu284Pro de novo	7m	Arm twitching, myoclonic, absence	MAE	Generalized epilepsy spectrum	Severe	Microcephaly (NP); Hypotonia; spasticity, regression (9y)	GSW (NP)	Normal	Pharmacoresistant
20 (M)		c. 851 T > C p.Leu284Pro de novo	6m	Absence, GTCS	EIEE	EDEE	Severe	Normal, prognathism	GSW (6m)	Normal	ETX
21 (F)		c.913G > A p.Ala305Thr de novo	NP	Atypical focal, migrating; GTCS	EIEE	EDEE	Severe	Congenital microcephaly; hypotonia, regression after 3m	Migrating PSW (3m)	Normal then atrophia	TPM, VPA, LEV
22 (M)	*GABRG2*	*c.844C > G* p.Pro282Ala de novo	Birth	Trembling, focal, myoclonic	EIEE	EDEE	Severe (died at 18y)	Hypotonia, dystonia, strabismus, nystagmus, dysmorphism	suppression burst (2m)	Atrophia, WM abnormality	Pharmacoresistant
Loop localization											
23 (F)	*GABRB2*	c.892A > G p.Lys298Glu de novo	3m	Atonic, GTCS, absence, alternating hemiplegia	GEFS+	Fever-related epilepsy	Mild	Ataxia	Focal SW, non-clinical frontal SW sleeping	Normal	BZD, VPA, ZNS
24 (F)		c.902A > G p.Tyr301Cys de novo	9m	Myoclonic, atonic, GTCS, atypical absence, photo S and sonosensibility	MAE	Generalized epilepsy spectrum	Moderate	Ataxia, hypotonia, macrosomia	Generalized ample PSW	Normal	LEV
25 (M)		c.908A > G p.Lys303Arg de novo	3d	Myoclonic, GTCS, tonic	EIEE	EDEE	Severe	Postnatal microcephaly, axial hypotonia, spasticity, strabismus, global growth delay at 7m	Suppression burst (3d)	WM hypersignal, cortical and CC atrophia	Vit. B6, PHB, TPM, ZNS, VPA

TABLE 1 (Continued)

Patient (gender)	Gene	Mutation inheritance	Age at onset	Seizure types	Epileptic phenotype	Diagnosis spectrum	ID	Clinical features	First EEG	Brain MRI	Effective drugs
26 (F)	*GABRB3*	c.911A > G p.Lys304Arg de novo	21d	Spasm, clonic,	EIEE	EDEE	Severe (died at 2m)	Macrocephaly, axial hypotonia, choreoathetoid movement, micro retrognathism	Hypsarrhythmia (21d)	Normal	Pharmacoresistant
27 (F)		c.1347_1350 delCAGA p.Arg450Glyfs*15 homozygous inherited	6d	GTCS, spasms	EIEE	EDEE	Severe	Axial hypotonia, aggressively, stereotypies	Suppression bust (6d)	Hypersignal WM	CLB, PHB, VPA
28 (F)			1d	GTCS, absence	EIEE	EDEE	Severe	Axial hypotonia, postnatal microcephaly, cleft palate	Suppression bust (2d)	NP	VGB, PHB
29 (F)			2d	Myoclonic, GTCS	EIEE	EDEE	Severe	Postnatal microcephaly, micro retrognathism	Normal (2d)	Hypersignal WM	PHB
30 (M)	*GABRG2*	c.967C > T p.Arg323Trp de novo	15m	Tonic clonic, atonic, atypical absence	MAE	Generalized epilepsy spectrum	Moderate	Hyperactivity, aggressivity, attention disorder	Multifocal SW (anterior and temporal) asymptomatic	Normal	LTG, ETX
31 (M)		*c.986G > A* p.Arg323Gln *Inherited*	9m	Focal hemicorporal, GTCS	Dravet syndrome	Fever-related epilepsy	Severe	No social interaction, stereotypies, ASD	Normal (9m)	Normal	CLB, VPA
32 (F)			8m	Atonic, absence, myoclonic; focal	Dravet syndrome	Fever-related epilepsy	Severe	Postnatal macrocephaly, stereotypies, ASD, dysmorphism, sleep trouble	Normal (8m)	Hydrocephalus	CLB, LTG
33 (M)		c.968G > A p.Arg323Gln de novo	10m	Focal, GTCS clonic, myoclonic-atonic	Incomplete Dravet	Fever-related epilepsy	Mild	Comportment disorders, attention disorders	Focal SW right parietal	Hypersignal WM	VPA, CLB KD
34 (F)		c.992A > G p.Tyr331Cys de novo	12m	GTCS	GEFS+	Fever-related epilepsy	Moderate	Postnatal macrocephaly, ataxia, visuomotor apraxia, choreoathetoid movement, obesity	Normal (1y)	Normal	VPA
35 (F)		c.1128 + 2 T > C *Inherited*	10m	Absences +/− clonic, ocular revulsions	GEFS+	Fever-related epilepsy	No	Comportment disorder	Multifocal SW (NP)	Normal	VPA, LEV
36 (M)			3y	Infantile spasms, tonic clonic, atonic, atypical absences	MAE	Generalized epilepsy spectrum	Mild to moderate	Concentration difficulties, ADHD	Multifocal SW, asymptomatic generalized PSW during sleep, normal (11y)	Normal	VPA, ETX, LTG, CLB, LEV
37 (M)			12m	Absences, GTCS	GEFS+	Fever-related epilepsy	Mild	Writing difficulties, aggressiveness	GSW	GSW(8y) normal after	VPA

Note: c.DNA newly reported mutation highlighted in gray. GABRA1: NM_000806.5; GABRG2: NM_000816.3; GABRB3: NM_000814.4; GABRB2: NM_021911.2.

Abbreviations: *Diagnosis and clinical features*: ADHD: attention-deficit/hyperactivity disorder, ASD: autism spectrum disorder, CC: corpus callosum, d: days, DS: Dravet syndrome, E: epilepsy, EDEE: early developmental epileptic encephalopathy, EOEE: early-onset epileptic encephalopathy, EEG: electroencephalography, EOEE: early-onset epileptic encephalopathy, FeS: febrile seizure, F: female, FS: focal seizure, GE: generalized epilepsy, GEFS+: generalized epilepsy with febrile seizure plus, GOR: gastroesophageal reflux, GSW: general spike waves, GTCS: generalized tonic-clonic seizure, GTCA: generalized tonic-clonic seizure alone, HC: head circumference, ID: intellectual disability, m: months, M: male, MAE: myoclonic-atonic epilepsy, NP: not precise, PSW: polyspike waves, photo S: photosensibility, sens.: sensitive, Sz: seizure, SD: standard deviation, SW: spike waves, TM: transmembrane domain, y: years, WM: white matter.*Treatment*: BZD: benzodiazepine, CBZ: carbamazepine, CLB: clobazam, ETX: ethosuximide, KD: ketogenic diet, LEV: levetiracetam, LTG: lamotrigine, OXC: oxcarbazepine, PHB: phenobarbital, PIR: piracetam, PMP: perampanel, TPM: topiramate, VGB: vigabatrin, Vit. B6: vitamin B6, VPA: valproic acid, ZNS: zonisamide.



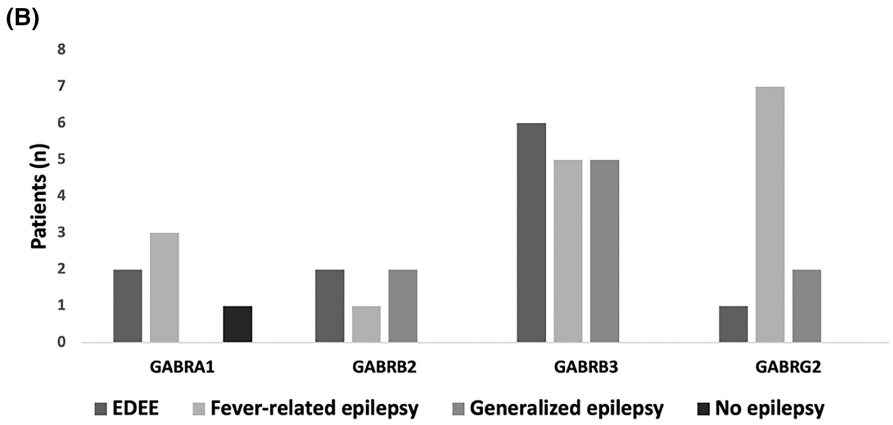


FIGURE 1 (A) Epileptic phenotype and (B) cognitive status related to the γ-aminobutyric acid (GABA) gene variant. EDEE: early developmental epileptic encephalopathy, ID: Intellectual disability

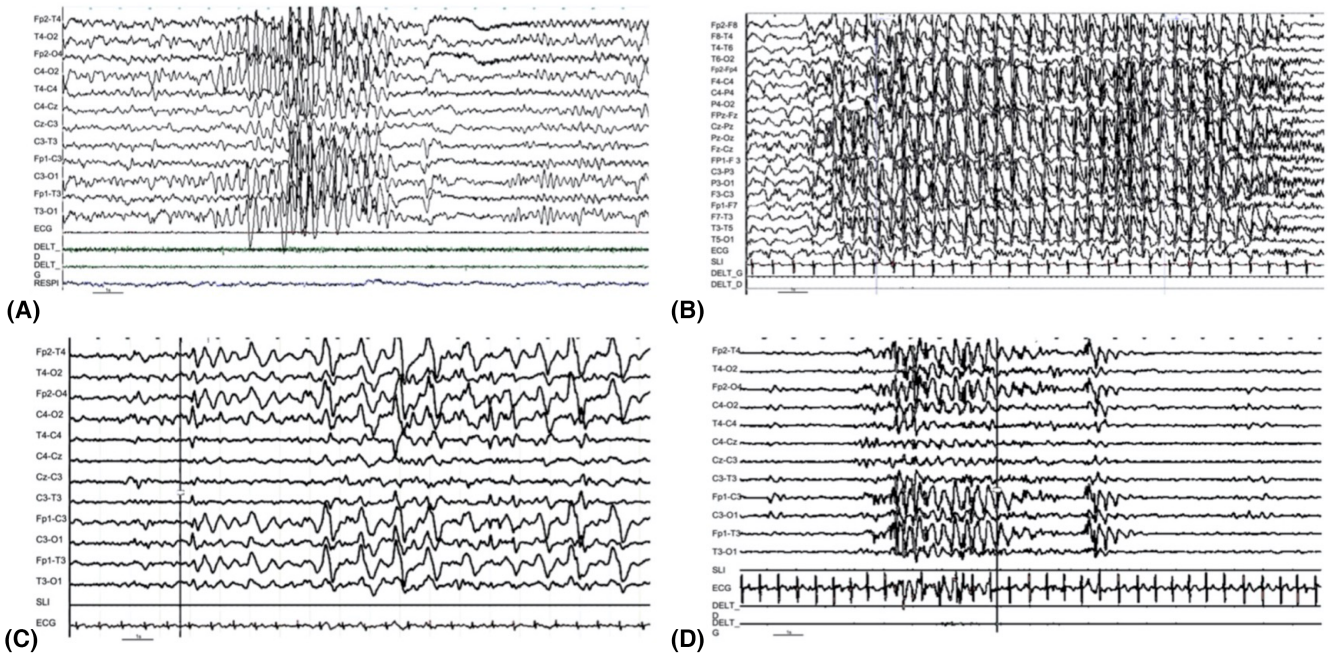


FIGURE 2 Interictal electroencephalography (EEG) recordings of patients with *GABRB3* variants. (A) Patient 12, wakefulness. Asymptomatic episode of theta rhythmic pattern. (B) Patient 9, sleep. (C) Patient 11, wakefulness, asymptomatic. (D) Patient 10, wakefulness, asymptomatic bursts of slow wave, predominantly anterior

of epilepsy, including myoclonic-atonic epilepsy ($n = 6$), myoclonic juvenile epilepsy ($n = 1$), and generalized tonic–clonic seizure alone ($n = 1$). Two patients had an unclassified epilepsy ($n = 2$). Most patients ($n = 6$) presented with generalized anomalies on the EEG, and two patients had an Angelman-like pattern on the EEG (Patients 5 and 9, Table 1). Five patients had moderate ID, three had mild ID, one had severe ID, and one had standard intelligence (Figure 1B). Lamotrigine was the most effective drug in seven patients and levetiracetam in three patients. Valproic acid and ethosuximide were rarely effective.

Patient 16 was not epileptic, had mild ID, and behavioral disorders, such as drooling, bruxism, and anxiety. At 2 years of age, the EEG was normal, whereas at 5 years, it was diffuse, high, and slow, with rhythmic spikes and waves. No correlation was observed between genetic pathogenic variants and the phenotype of the patient.

3.1.2 | Imaging characteristics

Thirty four of the 37 patients had brain magnetic resonance imaging (MRI). The majority (21/34) were reported as normal. The other displayed nonspecific abnormalities, including white matter hypersignal ($n = 7$), cortical atrophy ($n = 4$), corpus callosum anomalies ($n = 3$), ventriculomegaly ($n = 2$), and unilateral temporal arachnoid cyst ($n = 1$).

3.1.3 | Other neurological features

Seven patients had movement disorders, with stereotypies ($n = 5$), choreoathetosis movements ($n = 3$), tremor ($n = 2$), and dystonia ($n = 2$). Five patients had ataxic gait and three had spastic diplegia. No genotype–phenotype correlation was found in terms of motor or nonepileptic abnormalities.

Eleven patients had head circumference abnormalities: microcephaly ($n = 7$) and macrocephaly ($n = 4$). These abnormalities were observed particularly in patients carrying a mutation in *GABRB3* (8/16 patients: 5 with microcephaly and 3 with macrocephaly).

3.1.4 | Ophthalmological features

Two patients with variants in *GABRB3* had visual impairment. Patient 4 had a nystagmus with bilateral optic atrophy (confirmed with optic coherence tomography). Patient 9 had GEFS+ and mild ID, a nystagmus, bilateral hyperopia, reduced corrected visual acuity (4/10 for the right eye and 2.5/10 for the left), and a heterogeneous aspect of the peripheral retina. Two patients with the *GABRG2* variant had a visuospatial apraxia. In addition, three had nystagmus and three had strabismus; no specific gene variant association was found.

3.1.5 | Dysmorphism

Eight patients were described with particular morphological facial features, particularly patients carrying a variant in *GABRB3* (four patients). These morphological features affected the lower and middle parts of the face and included high-arched palate, chin anomalies (microretrognathism, prognathism), and cleft palate.

3.2 | Molecular results

3.2.1 | Type of variants

In our cohort of 37 unreported patients, 27 different pathogenic $GABA_A$-receptor subunits were noted: 5 in *GABRA1*, 5 in *GABRB2*, 11 in *GABRB3*, and 6 in *GABRG2;* 17 variants were unpublished (4 in *GABRA1*, 4 in *GABRB2*, 5 in *GABRB3*, and 4 in *GABRG2*). Variant types included 23 missense, 2 frameshifts, 1 nonsense, and 1 intronic variant. Variants were heterozygous in 34 cases and homozygous in 3 siblings in a consanguineous pedigree.

3.2.2 | Inheritance

The mutations observed were heterozygous de novo in 25 patients and inherited in 12 patients (Figure S1). Three patients from the same family (Patients 27, 28, and 29) had a homozygous *GABRB3* pathogenic variant associated with a particularly severe phenotype of EDEE, with seizures beginning from 1 to 6 days of age. In this consanguineous pedigree, mutations were inherited from their heterozygous parents with milder phenotypes. Both parents displayed mild ID, and the mother exhibited medication-sensitive epilepsy during childhood. Although this variant has never been reported, it has been considered pathogenic. The other inherited cases are detailed in the supplemental data.

3.2.3 | Variant location

We analyzed the identified variants based on their localization within the GABA receptor protein: NT as N-terminus, TM as transmembrane domain, and the loop domain. Ten variants were located in the extracellular NT of the protein, eight in TM domains (two in TM1, five in TM2, and one in TM3), and nine in loops (seven in loop TM2-3 and two in

loop TM3-4) (Figure 3). The variants in the intracellular domain (C-term) were rare in our cohort, as in the literature.

3.3 | Literature review and phenotype–genotype correlation

We reviewed 402 previously published cases: 71 patients with a mutation in *GABRA1*, 28 in *GABRB2*, 133 in *GABRB3*, and 170 in *GABRG2*. The distribution according to the phenotype was as follows: 68 reported mutations had an undetailed clinical description, and 77 reported mutations had a phenotype that did not fit into any of the groups we described (20%). Some mutations were reported without clinical description. Finally, the clinical and genetic data were available in 334 cases, and 257 patients were analyzed.

Most of the patients were epileptic, but eight had ID without epilepsy (except for one patient without ID or epilepsy, the mother of three affected children).

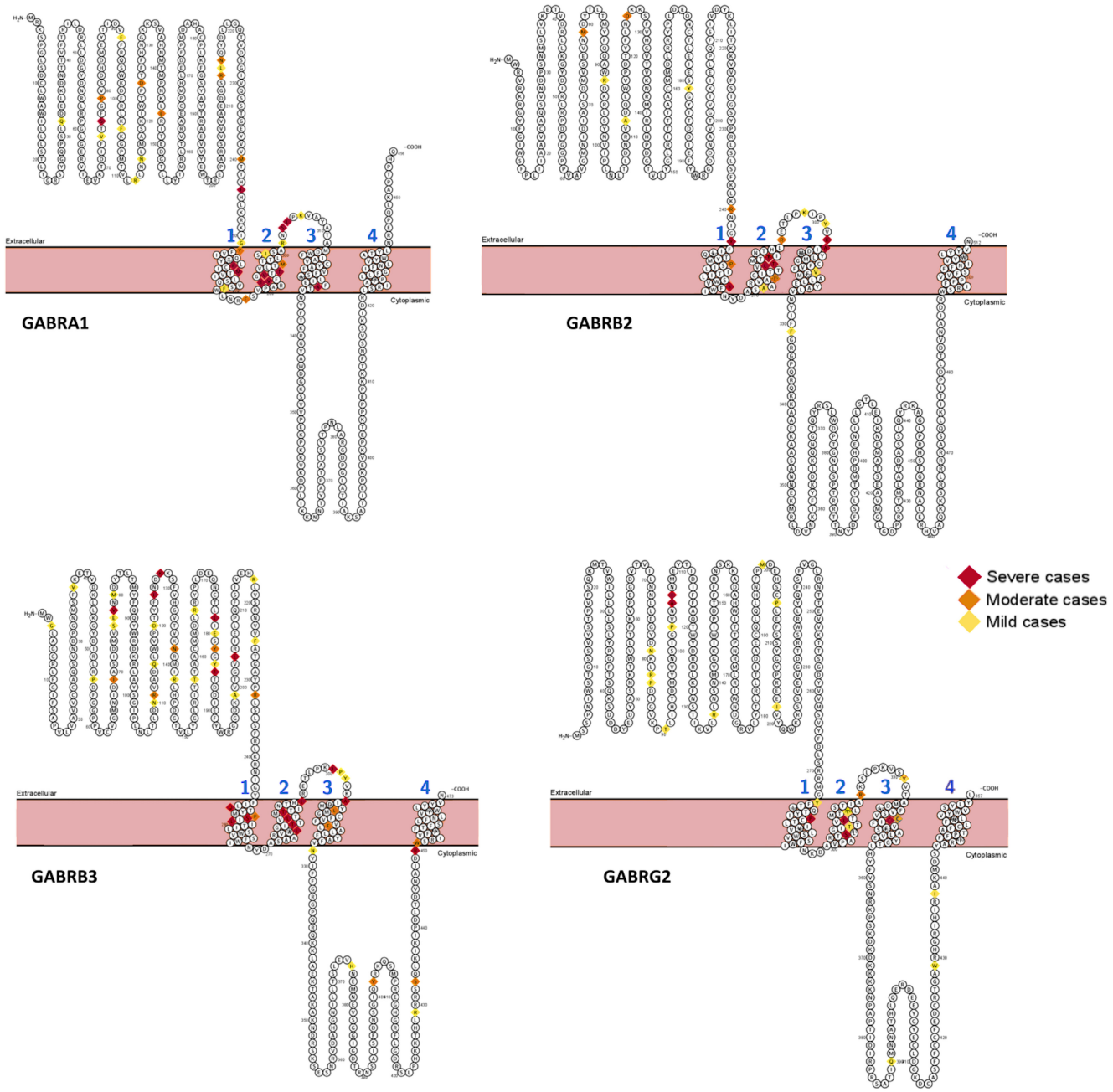


FIGURE 3 Location of pathogenic variants identified in *GABRA1, GABRB2, GABRB3*, and *GABRG2* protein classified by the intellectual disability severity of the reported associated phenotype. Variants for which no information is available in the literature regarding the phenotype are not noted

Fifty-two mutations were recurrent (15 in *GABRA1*, 5 in *GABRB2*, 10 in *GABRG2*, and 22 in *GABRB3*) concerning 185 patients and 129 families (25 localized in the NT domain and 27 in TM or loop domains; Figure S2). Among these recurrent mutations, 18 were reported in more than three families (Figure S2); c.968G > A in *GABRG2* was reported mostly in 13 different families associated with a milder phenotype.

We observed an overrepresentation of severe cognitive impairment in patients with variants located in the TM region. To analyze the potential genotype–phenotype correlation according to protein location, we compared the clinical characteristics of patients in the cohort ($n = 37$) and those reported in the literature ($n = 402$), depending on the location of variants (Table S1 and Appendix S1). We selected three relevant and available clinical data: age at epilepsy onset, epilepsy severity, and ID severity.

Severe ID was noted in 39% of patients in our cohort and 38% of cases in the literature (Table S1). The proportion of patients with severe ID was enriched among patients with variants in TM (87%), higher than in cases with variants localized to the loop (38%) or NT (22%) (χ^2 (2)=16.9, $p < .001$, in our cohort and χ^2 (2)=42.6, $p < .001$ in the literature) (Figures 4–5).

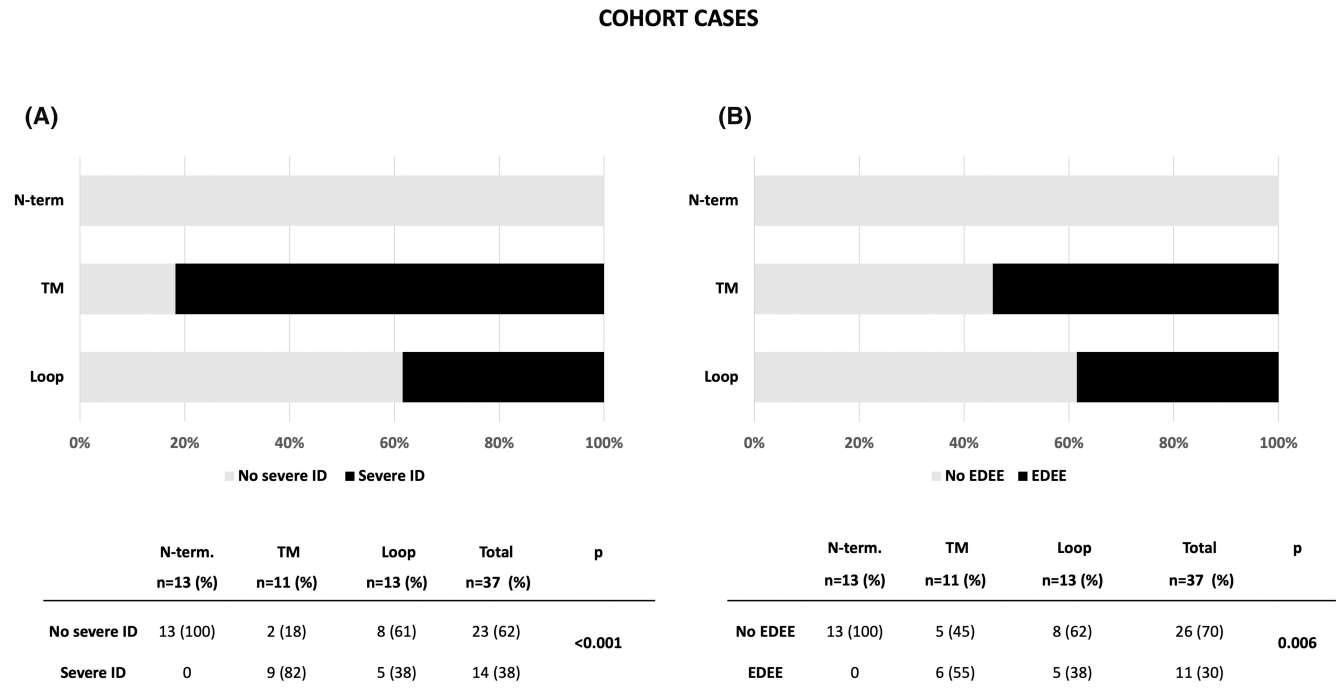
In our cohort, more than half of patients with EDEE had TM variants (55%). Indeed, the TM variants were more often associated with EDEE; 30% in our cohort (χ^2 (2)=10.2, $p = .006$) and 25% in the literature (χ^2 (2)=27.8, $p < .001$) (Figures 4–5).

However, no correlation was observed between variant location and abnormal movements, behavioral disorders, or abnormalities on brain MRI.

4 | DISCUSSION

Here, we have described a cohort of 37 patients with epilepsy and/or ID, carrying pathogenic variants in several genes (*GABRA1, GABRB2, GABRB3*, and *GABRG2*) encoding most common $GABA_A$-receptor subunits.

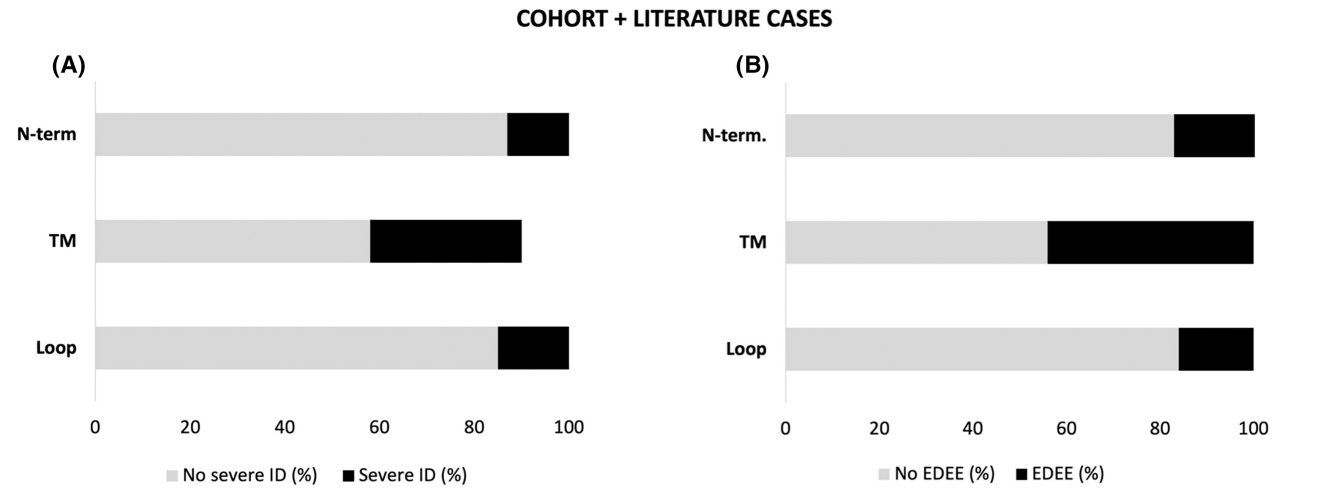
We reported three main epilepsy phenotypes of almost equal quantitative importance: a fever-relative epilepsy spectrum (GEFS+ and DS), an early-onset DEE spectrum, and a genetic generalized epilepsy spectrum, represented primarily by myoclonic-astatic epilepsy. None of these phenotypes was associated with a given gene (i.e., a selective subunit dysfunction), and in contrast, our study emphasizes the overlapping phenotypes of patients with $GABA_A$-receptor subunit variants with regard to epilepsy subtypes, ID severity, neurological findings, and brain imaging. Studies of recurrent variants highlight the complexity of a genotype–phenotype correlation approach, particularly regarding genes encoding receptors and channels.[27] Indeed, for example, patients carrying the mutation c.968G>A; p.Arg323Gln of *GABRG2* had either



	N-term. n=13 (%)	TM n=11 (%)	Loop n=13 (%)	Total n=37 (%)	p
No severe ID	13 (100)	2 (18)	8 (61)	23 (62)	<0.001
Severe ID	0	9 (82)	5 (38)	14 (38)	

	N-term. n=13 (%)	TM n=11 (%)	Loop n=13 (%)	Total n=37 (%)	p
No EDEE	13 (100)	5 (45)	8 (62)	26 (70)	0.006
EDEE	0	6 (55)	5 (38)	11 (30)	

FIGURE 4 Correlation between phenotype (A. severe ID, B. EDEE) and location of mutated GABA-R protein domains in our cohort ($n = 37$). ID: intellectual disability, EDEE: early developmental epileptic encephalopathy, pathogenic variant location: N-term, loop, and TM (transmembrane).



	N-term. n=239 (%)	TM n=105 (%)	Loop n=76 (%)	Total n=420 (%)	p
No severe ID	208 (87)	68 (58)	65 (85)	341 (82)	<0.001
Severe ID	31 (13)	37 (32)	11 (15)	79 (18)	

	N-term. n=239 (%)	TM n=116 (%)	Loop n=76 (%)	Total N=431(%)	p
No EDEE	198 (83)	65 (56)	64 (84)	327 (76)	<0.001
EDEE	41 (17)	51 (44)	12 (16)	104 (24)	

FIGURE 5 Correlation between phenotype (A. severe ID and B. EDEE) and location of mutated GABA-R protein domains in our cohort and literature cases. ID: Intellectual disability, EDEE: Early developmental epileptic encephalopathy, pathogenic variant location: N-term, loop, and TM (transmembrane)

DS (Patients 31 and 32), a GEFS+ with mild cognitive impairment (Patients 33 and 31–32's mother), a GEFS+ with normal cognitive status,[28] childhood epilepsy with centrotemporal spikes,[29] or early-onset epileptic encephalopathy with severe ID.[15] This mutation is highly recurrent and has been found in 13 different families from the literature (Figure S2). Other recurrent mutations were associated with a more homogeneous phenotype; for example, the p.Lys303Arg in *GABRB2* was always associated with EDEE.[30] Most of the inherited variants reported here led to a homogeneous epileptic phenotype in a given family when they were heterozygous. This was not always the case regarding the degree of ID within a given family (Figure S1). Finally, we did not find any correlation between antiseizure medication effectiveness and a given gene or a specific subunit localization.

We found a homozygous frameshift variant of *GABRB3* in three consanguineous siblings with an EDEE and a suppression-burst EEG pattern. These patients had a highly severe phenotype, and their heterozygous parents had a mild one. In the present case, the variant was located in the last exon of the gene and was probably not subject to nonsense-mediated decay. It is then likely that a transcript still exists in the patients' cells that is different from the normal transcript in the C-terminal region. A functional study would be particularly interesting in this case. It is likely that biallelic mutations of GABA receptor subunits will be further described in the future because many mutations are not associated with a highly severe phenotype at the heterozygous state. Genes coding for the GABA receptor subunits join the long list of genes involved in both recessive and dominant diseases.

Disease-causing variants were located in critical functional protein domains, including the extracellular NT domain (12 cases); the extracellular loop between TM2 and TM3 (9 cases); and TM2, which forms the $GABA_A$ channel pore (6 cases) (Figure 3, Table S1 and (Ref. 10)). We observed a significant correlation between ID degree, epilepsy severity, and variant localization through this cohort but also among the patients published to date (402 cases). Patients with NT variants presented with milder epilepsy and ID than patients with variants in other regions of the protein, regardless of the mutated subunit, whereas variants in TM domains had the most severe phenotype in terms of epilepsy and ID. This correlation has been reported recently for *GABRB2*[13] and *GABRB3* cohorts.[25] In this recent report, milder phenotypes (generalized epilepsy associated with mild to moderate ID) were associated with a mutation in the extracellular domain, whereas patients with early-onset epilepsy and severe ID had a mutation in TM or the extracellular domain. Our findings expand this correlation to three other $GABA_A$-receptor subunit genes.

We found missense variants in 29 patients and protein truncation variants in 7 patients. Protein truncation most likely leads to a loss of function and may reduce inhibitory phasic GABAergic transmission.[13,14,31,32] Missense variants may either be a loss or a gain of function.[26] In *GABRB3*, gain-of-function variants were associated with early-onset epilepsy, more severe intellectual disability, hypotonia, and more pharmacoresistance, whereas loss of function mutations were most frequently related to a milder phenotype and febrile seizures.[26] Gain-of-function variants were mostly localized in the TM domain and associated with the most severe phenotype. It is then possible that the variants localized in the TM domain could be linked with gain of function, whatever the subunit. This finding also suggests that within the $GABA_A$ receptor gene, the location of the mutation is more important than the time of expression of the gene during development in terms of predicting the phenotype. Although it is relatively easy to make the link between loss of function of GABAergic receptors and epilepsy, the association is less clear for gain-of-function mutations. It is possible that epilepsy is not the direct consequence of the mutation at the time of onset but the result of abnormal early developmental brain activities (due to the mutation). As suspected for *KCNQ2*-related DEEs, epilepsy and ID would be the late consequence of alterations in network activities induced earlier (and transiently) by the mutation.[33]

Among DEE patients, we often found an EEG pattern similar to that observed in Angelman syndrome, made of irregular bursts of ample slow/acute activity in frontal regions (Figure 2). This pattern has also been found in *Gabrb3* +/− and *Gabrb3* −/− mice[34] and may be a potential biomarker of a GABAergic dysfunction.[16]

A number of ASMs (vigabatrin, topiramate, valproic acid, cenobamate) are known to affect GABA receptors via different mechanisms (GABA receptor activation, reuptake inhibition, neurotransmitter breakdown inhibition, and so on).[35] The effect of these molecules has already been reported, with inconsistent consequences.[15,36–39] The existence of gain-of-function mutations may explain the lack of efficacy or the worsening effect of these ASMs and the potential efficacy of sodium channel blockers in some cases.[40–42] Although interpreted with substantial caution, the most effective ASMs were different within the three groups in our cohort: valproic acid in the fever-sensitive group, phenobarbital in the DEE group, and lamotrigine in the GGE group. It is important to note that these treatments were not chosen according to the mutation and were not modified after the molecular results had been obtained. It would be of significant interest to test the potential relationship between functional consequences and the effect of a given ASM.

Here, we reported variants in the most common $GABA_A$-receptor subunits in the human brain: ~60% of all $GABA_A$-receptor subunits have the combination α1β2γ2, and 15%–20% have the combination α2β3γ2. However, several other genes code for other rare subunits of this receptor (19 in total). Mutations in some of these genes have been identified in patients with similar phenotypes, with epilepsy (including EDEE) and ID. However, the conclusions drawn here cannot yet be generalized for all $GABA_A$-receptor subunits, and further work is still required.

5 | CONCLUSION

We have described the phenotypes associated with variants in the four most common subunits of $GABA_A$ receptors in humans—*GABRA1, GABRB2, GABRB3*, and *GABRG2*—so that we could determine the epileptic and developmental features and identify genotype–phenotype associations. From our observations, no genotype–phenotype associations could be evidenced among $GABA_A$-receptor subunits regarding epilepsy, response to treatment, ID severity, neurological findings, and brain imaging. However, a significant genotype–phenotype correlation was found regarding the variant localization within the $GABA_A$-receptor subunit protein domains. Patients with an NT pathogenic variant showed significantly milder phenotype (milder ID and less severe epilepsy) compared to variants in TM domains (more severe) or other locations (intermediate). These results have also been confirmed by a comprehensive literature analysis of 402 reported cases. Then, in the case of finding a mutation of a $GABA_A$-receptor subunit in a patient, a rapid assessment of the mutation's location may be helpful to better anticipate the disease burden.

AUTHOR CONTRIBUTIONS

Pierre-Yves Maillard, Sarah Baer, Béatrice Desnous, Anne de Saint Martin, and Mathieu Milh contributed to study design and conceptualization, data collection, analysis, interpretation of data, and the original draft of the manuscript. Élise Schaefer, Caroline Lacoste, Salima El Chehadeh, Amélie Piton, Giulia Barcia, Gaëtan Lesca, Véronique Paquis-Flucklinger, and Laurent Villard performed the genetic analysis and interpretation. Nathalie Villeneuve, Anne Lépine, Alexandre Fabre, Caroline Lacoste, Salima El Chehadeh, Amélie Piton, Louise Frances Porter, Caroline Perriard, Marie-Thérèse Abi Wardé, Marie-Aude Spitz, Vincent Laugel, Gaëtan Lesca, Audrey Putoux, Dorothée ville, Cyril Mignot, Delphine Héron, Rima Nabbout, Marlène Rio, Agathe Roubertie, Pierre Meyer, Olivier Patat, Jérémie Lefranc, Marion Gerard, and Julietta de Bellescize contributed to data

collection and interpretation, and to revision of the manuscript for intellectual content. Statistical analysis was done by Béatrice Desnous.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest in line with this study to declare.

ORCID

Pierre-Yves Maillard https://orcid.org/0000-0002-7766-1072
Sarah Baer https://orcid.org/0000-0003-4951-2552
Béatrice Desnous https://orcid.org/0000-0003-2571-8564
Gaëtan Lesca https://orcid.org/0000-0001-7691-9492
Dorothée Ville https://orcid.org/0000-0001-9432-4409
Rima Nabbout https://orcid.org/0000-0001-5877-4074
Laurent Villard https://orcid.org/0000-0001-6657-5008
Mathieu Milh https://orcid.org/0000-0002-2454-8543

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SUPPORTING INFORMATION

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

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