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ORIGINAL ARTICLE

Seizure features and outcomes in 50 children with GATOR1 variants: A retrospective study, more favorable for epilepsy surgery

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Abstract

Objectives: To summarize the clinical features of epilepsy related to *DEPDC5*, *NPRL2*, and *NPRL3* genes encoding the GATOR1 complex in children and to evaluate the factors affecting the prognosis of these epilepsies.

Methods: In this retrospective study, we reviewed the clinical and genetic characteristics of children with epilepsy related to GATOR1 variants who were admitted to the Peking University First Hospital between January 2016 and December 2021. Potential prognostic factors were assessed by comparing children with and without ongoing seizures.

Results: Fifty probands, including 31 boys and 19 girls were recruited. The median age at onset of epilepsy was 4 months, and 64% of patients had early-onset epilepsy (≤1 year). The most frequent epileptic seizure type was focal seizure (86%). Among the 50 patients, only six were with de novo variants. According to the novel classification framework for GATOR1 variants, 36 patients were with pathogenic variants and 14 with likely pathogenic variants. *DEPDC5* variants were found in 37 patients, *NPRL3* in 9, and *NPRL2* in 4. The phenotype was similar among the probands, with variants in *DEPDC5*, *NRPL2*, or *NPRL3*. 76% (38/50) of epilepsy related to GATOR1 variants was neuroimaging positive, including brain MRI positive in 31 patients, and MRI combined F-18-fluorodeoxyglucose positron emission tomography positive in the other seven patients. Twenty-seven patients underwent epilepsy surgery. In total, after initial antiseizure medications alone, 92% (46/50) of patients were drug-resistant epilepsies, only 8% (4/50) of the

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probands became seizure-free but seizure-free (≥6 m) occurred in 92.6% (25/27) of patients with drug-resistant epilepsy after epilepsy surgery at the last follow-up. Patients undergoing epilepsy surgery had better epilepsy prognosis.

Significance: Epilepsy related to GATOR1 variants had high possibility to be drug-resistant epilepsy and to have positive neuroimaging finding. Epilepsy surgery is the only favorable factor for better seizure prognosis in this kind epilepsy.

KEYWORDS

children, epilepsy surgery, GATOR1-related epilepsies, MRI-negative, seizure outcomes

JEL CLASSIFICATION

refractory epilepsy, genetic epilepsy, Epilepsy surgery

1 | INTRODUCTION

The GAP activity towards rags complex 1 (GATOR1) complex, comprising DEPDC5 (DEP domain-containing protein 5), NPPL2, and NPRL3 (nitrogen permease regulator-like 2 and 3), is an amino acid-sensing negative regulator of the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway,[1] which is a ubiquitous regulator of cell metabolism, proliferation, and protein synthesis. In the brain, it is essential for neuronal cell proliferation, differentiation, and the formation of neural network.[2,3] In 2013, *DEPDC5* variants were first shown to be associated with familial focal epilepsy (FFE).[4,5] Additional studies revealed that the phenotype of individuals with *NPRL2* or *NPRL3* variants was similar to the DPEDC5 phenotype, defined as the "GATOR1 phenotype".[6,7] It comprises a series of focal epilepsy syndromes, including FFE with variable foci, lateral temporal lobe epilepsy, and autosomal dominant sleep-related hypermotor epilepsy. Rolandic epilepsy and West syndrome have also been reported as part of the phenotype spectrum.[8–10] The severity of symptoms varies greatly, ranging from asymptomatic carriers to drug-resistant epilepsy and sudden unexpected death related to epilepsy. Nearly half of the patients with GATOR1 variants are drug-resistant.[6] Notably, malformations of cortical development (MCD), which is a common cause of drug resistance and accounts for 19.8% of patients undergoing epilepsy surgery,[11] was noticed in some individuals with GATOR1 variants. Favorable epilepsy surgery outcomes were reported in these patients, nearly 50%-60%[6,12] of whom achieved seizure-free after surgery.

We explored the prognostic factors of whether seizures were controlled at the last follow-up, especially the influence of epilepsy surgery on prognosis. In this study, we reviewed the clinical and genetic data of 50 Chinese children with epilepsy caused by the GATOR1 variants.

Key Points

- Epilepsy related to GATOR1 variants had high possibility to be drug-resistant
- Epilepsy surgery results in the significant improvement of seizure outcomes for patients with GATOR1 variants
- More presurgical investigations need to be conducted for MRI negativity patients to confirm epileptic foci

Furthermore, we investigated the association between the phenotype and genotype in GATOR1-related epilepsy.

2 | MATERIALS AND METHODS

2.1 | Ethics statements

This study was approved by the Institutional Review Board of the Ethics Committee of Peking University First Hospital (2015-870). Informed consent was obtained from the parents of all the children.

2.2 | Patient selection

We retrospectively selected patients with GATOR1 variants at the Pediatric Epilepsy Center of Peking University First Hospital between January 2016 and December 2021, including individuals who underwent presurgical evaluation of epilepsy. The inclusion criteria were as follows: (a) age at seizure onset ≤18 years, (b) absence of defined genetic or nongenetic causes other than the GATOR1

variant, and (c) follow-up for at least 6 months after surgery for patients with epilepsy surgery. Patients with incomplete data or those who carried variants with uncertain significance (VUS) according to a novel classification framework, which was proposed by Baldassari et al.[6] and specifically adapted to GATOR1 variants, were excluded from the cohort. Of 77 patients with GATOR1 variants, 50 were recruited and 27 were excluded from the cohort, including four patients with incomplete data, one patient with a history of Japanese encephalitis, and 22 patients with the VUS variants.

2.3 | Clinical features

Presurgical information was reviewed for all patients, including gender, age at seizure onset, seizure types, number of anti-seizure medications (ASMs), family history, and neuroimaging findings. The seizure types and epilepsy syndromes were classified according to the International League Against Epilepsy (ILAE) classification.[13,14] Infantile epileptic spasm syndrome (IESS) is characterized by epileptic spasms, regardless of hypsarrhythmia and neurodevelopmental delay. IESS encompasses West syndrome, which refers to the triad of epileptic spasms, hypsarrhythmia, and neurodevelopmental delay. For patients with drug-resistant epilepsy, which is defined as failure of achievement of sustained seizure freedom after two kinds of tolerated, appropriately chosen and used ASMs, presurgical evaluations were performed when necessary. It included brain 3.0 T magnetic resonance imaging (MRI), F-18-fluorodeoxyglucose positron emission tomography (FDG-PET), long-term video electroencephalogram, and neuropsychological assessments. For patients who underwent epilepsy surgery, we also collected surgery-related data, including the duration of epilepsy prior to surgery, type of surgical procedure (classified as lobectomy, hemispherotomy, and functional hemispherectomy or radiofrequency ablation), histopathological diagnosis, seizure outcome classified using the Engel Epilepsy surgery Outcome Scale,[15] and length of follow-up.

2.4 | Genetic studies

Trio Whole genome sequencing was performed in two patients, trio whole exome sequencing (WES) in 32 patients, proband-only WES in seven patients, and epilepsy gene panel in nine patients. And for patients receiving proband-only WES or epilepsy gene panel, the GATOR1 variant has been verified by Sanger sequencing in the probands and their patients. We collected the necessary information on GATOR1 variants, including genomic position (hg19), reference allele, alternative allele, and its inheritance information. Then, we reannotated and reclassified them according to the ACMG guidelines[16] and the novel classification framework proposed by Baldassari et al.[6]

Available variant call format (VCF) files of 16 patients were collected from gene-sequencing centers, and variants were reannotated and filtered using ANNOVAR,[17] based on the frequency and functional changes. For the rare variants (minimum allele frequency <1% in any of the gnomAD, Exac, and 1000 Genomes Project databases), which were also identified as pathogenic by in silico methods, we determined their pathogenicity according to the ACMG guidelines[16] to ensure that there were no other causative mutations related to epilepsy.

2.5 | Statistical analysis

Patient characteristics are summarized using descriptive analysis. Categorical variables are summarized as numbers and percentages of the total number of patients in each category, including variant information, epilepsy phenotype, cognitive comorbidities, family history, neuroimaging findings, type of surgical procedure, histopathological diagnosis, and seizure outcome. Continuous variables, which were all non-normally distributed variables in our cohort, including age at epilepsy onset, epileptic duration, number of ASMs, and length of follow-up, are described as medians and ranges.

Factors were collected to analyze their correlation with seizure outcomes. Continuous variables were compared using the Mann–Whitney *U* test, and the Fisher exact and chi-square tests were used to compare categorical variables. We also analyzed the correlation between seizure outcome and the distribution of variants of the different GATOR1 domains. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SPSS for Windows (version 24.0; IBM Corp.).

3 | RESULTS

3.1 | Patients' demographic and clinical characteristics

The 50 probands included 31 boys and 19 girls. The median age at the onset of epilepsy was 4 (range, 0-124) months, and 64% (32/50) had early-onset epilepsy (≤1 year). In total, 24% (12/50) of the probands had a family history of epilepsy. The most frequent epileptic

seizure type was focal seizure (86%), followed by epileptic spasm (40%). Neuroimaging examination was performed on each proband, including 50 probands with 3.0 T MRI and 43 probands with FDG-PET. Totally, 38 patients had positive neuroimaging findings. Thirty-one patients were found by MRI alone, and seven patients by combined brain FDG-PET with MRI. After a comprehensive preoperative evaluation in 43 patients, epileptic surgeries were performed in 27 patients, 22 with lobar resection, four with hemispherotomy, and one with radiofrequency thermocoagulation. All of them had positive neuroimaging findings, with 20 identified by MRI alone. Stereo electroencephalography (sEEG) was performed in four patients to confirm the epileptic foci. Noteworthily, among the patients with positive neuroimaging findings, 11 patients have not yet undergone epilepsy surgery because of various reasons, including achieving seizure-free by the treatment of ASMs alone, failing the preoperative evaluation because of the electroclinically and neuroimaging inconsistency and so on. The baseline data were summarized in Tables 1 and 2, and the detailed information of these 50 patients was in Table S4.

3.2 | Seizure outcomes and prognostic factors

After initial ASMs treatment, 92% (46/50) were drug-resistant epilepsies, and only 8% (4/50) of the probands achieved seizure-free. Among the 27 patients who underwent epilepsy surgery, 92.6% (25/27) of them were seizure-free (Engel I), and 7.4% (2/27) were almost seizure-free (Engel II) at the last follow-up. The follow-up time was at least 1 year after surgery (range, 1-5 years). Histological examinations confirmed FCD in 20 probands, including three with FCD Ia, two with FCD Ib, and 15 with FCD IIa. Mild MCD (mMCD) was reported in the other seven patients.

We evaluated the factors affecting the prognosis of these patients (Table 3). Compared with patients with seizure-ongoing, the patients with seizure-free had a significantly higher percentage of neuroimaging abnormality ($P < 0.001$), and higher percentage of undergoing epilepsy surgery ($P < 0.001$). Other factors, including gender, epilespy phenotype, family history, and mutated genes, were without statistically significant. Among patients with positive neuroimaging findings, patients with seizure-free also had more percentage of undergoing epilepsy surgery ($P < 0.001$, Table S1). For 43 patients with drug-resistant epilepsy who also received preoperative evaluation, patients with seizure-free all underwent epilepsy surgery and none achieved seizure-free by continued ASMs treatments alone ($P < 0.001$, Table S2).

3.3 | Genetic results and genotype–phenotype correlation

In total, 49 variants were identified in this study. Two patients carried the same variant of *NPRL3* p.R424*. Among them, 37 variants were novel mutations and the other 12 variants had been previously reported. *DEPDC5* variants were found in 37 patients (74%), *NPRL3* in 9 (18%), and *NPRL2* in 4 (8%). The phenotype was similar among the probands with variants in *DEPDC5*, *NRPL2*, or *NPRL3*. Among these, only 6 (12%) variants were de novo, 40 from asymptomatic parents, and four from parents with epilepsy or febrile seizures. Of all 49 distinct variants, 27 were frameshift or nonsense variants, five were canonical splice-site variants, three were copy number variants, 13 were missense variants and the other one was splice-region variant.

According to the new ACMG guidelines, a total of 70% (35/50) were classified as pathogenic (P) or likely pathogenic (LP), and the others were VUS. Based on the novel classification framework for GATOR1 variants,[6] 36 variants, including all ACMG P/LP variants and one VUS variants (*DEPDC5* p.F1472fs), were reclassified as P. And the other 14 variants were reclassified as LP. When comparing the differences between the ACMG P/LP and ACMG VUS groups, there were no statistically significant differences in phenotype and seizure outcome (Table S3). We further mapped the locations of the missense variant sites on *DEPDC5*, *NPRL2*, and *NPRL3* (Figure 1), which were distributed throughout the entire protein structure. We did not find any significant clustering of any of the main domains.

4 | DISCUSSION

Recent studies have reported that the GATOR1 complex is the most frequently mutated gene in familial and sporadic focal epilepsies, and nearly half of the patients with GATOR1-related epilepsy have drug-resistant epilepsy. Although this was a retrospective single-center study, it was the largest cohort of probands with GATOR1 variants to date. We found that patients with GATOR1 variants had a higher possibility (92%) to be drug-resistant epilepsy in our cohort but had a significantly higher seizure-free rate after epilepsy surgery.

We reviewed the clinical features of 50 probands with GATOR1 variants collected at our institution. Among GATOR1 variants, *DEPDC5* accounted for 74%, which might be related to the fact that it is the most frequent gene because of its longer transcript than *NPRL2*/3.[18] In this cohort, GATOR1 variants showed a similar phenotype, which confirmed the findings from previous studies that

TABLE 1 Demographic and clinical features of the 50 probands with GATOR1 variants.

	GATOR1	*DEPDC5*	*NPRL2*	*NPRL3*	*P*-value
Number of probands	50	37/50 (74%)	4/50 (8%)	9/50 (18%)	
Gender (male: female)	31:19	21:16	4:0	6:3	0.30
Age at seizure onset (m)	4 (0-124) (64%, <12 m)	4 (0-124) (59.5%, <12 m)	0.85 (0.17-2) (100%, <12 m)	5 (0.17-34) (66.7%, <12 m)	0.09
Seizure types					
Focal	43/50 (86%)	31/37 (83.8%)	4/4 (100%)	8/9 (88.9%)	1.00
Spasm	20/50 (40%)	18/37 (48.6%)	1/4 (25%)	1/9 (11.1%)	0.10
>2 seizure types	21/50 (42%)	17/37 (45.9%)	2/4 (50%)	2/9(22.2%)	0.48
Epilepsy types					
IESS	19/50 (38%)	17/37 (45.9%)	1/4 (25%)	1/9 (11.1%)	0.13
West syndrome	6/50 (12%)	6/37 (16.2%)	0/4 (0%)	0/9 (0%)	0.45
Family history of epilepsy	12/50 (24%)	11/37 (29.7%)	0/4 (0%)	1/9(11.1%)	0.38
Cognitive comorbidities					
Delay	31/50 (62%)	22/37 (59.5%)	3/4 (75%)	6/9 (66.7%)	1.00
Novel classification framework					0.67
P	36/50 (72%)	28/37 (75.7%)	3/4 (75%)	5/9 (55.6%)	
LP	14/50 (28%)	9/37 (24.3%)	1/4 (25%)	4/9 (44.4%)	
Inheritance					0.45
De novo	6/50 (12%)	6/37 (16.2%)	0/4 (0%)	0/9 (0%)	
Inherited	44/50 (88%)	31/37 (83.8%)	4/4 (100%)	9/9 (100%)	
Asymptomatic parents	40/50 (80%)	27/37 (73.0%)	4/4 (100%)	9/9 (100%)	
Neuroimaging					
Positive MRI findings	31/50 (62%)	20/37 (54.1%)	4/4 (100%)	7/9 (77.8%)	0.13
Positive MRI + FDG-PET findings	38/50 (76%)	26/37 (70.3%)	4/4 (100%)	8/9 (88.9%)	0.39
MCD	38/50 (76%)	26/37 (70.3%)	4/4 (100%)	8/9 (88.9%)	0.39
FCD	25/50 (50%)	15/37 (40.5%)	3/4 (75%)	7/9 (77.8%)	0.07
ASMs	4 (1-8)	4 (1-8)	4.5 (4-6)	4 (2-8)	0.23
Epilepsy surgery	27/50 (54%)	18/37 (48.6%)	4/4 (100%)	5/9 (55.6%)	0.19
Seizure-free	29/50 (58%)	21/37 (56.8%)	4/4 (100%)	4/9 (44.4%)	0.16
Medical	4/50 (4%)	4/37 (10.8%)	0/4 (0%)	0/9 (0%)	
Surgical	25/50 (54%)	17/37 (45.9%)	4/4 (100%)	4/9 (44.4%)	

Abbreviations: ASM, antiseizure medications; FCD, focal cortical dysplasia; FDG-PET, F-18-fluorodeoxyglucose positron emission tomography; IESS, infantile epileptic spasm syndrome; LP, likely pathogenic; MCD, malformations of cortical development; MRI, magnetic resonance imaging; P, pathogenic; IESS encompasses West syndrome.

proposed the "GATOR1 phenotype".[6,19] Focal epilepsy was the most common epilepsy phenotype, followed by epileptic spasms. IESS was the most common epilepsy syndrome in this cohort, occurring in 38% of patients. Cognitive impairment was reported in 62% of probands, which is higher than that in a previous study (7%-40%).[20] Drug resistance was observed in 92% of probands, which is higher than that reported in previous studies (52%-54%).[6,20] The higher percentage of neuropsychiatric comorbidities and drug resistance may be explained by the presence of more probands with early-onset epilepsy (64%) in this study. This selective bias might reflect the fact that patients with more severe phenotypes and younger seizure onset age preferred to visit advanced professional hospitals and to receive a molecular diagnosis.

In this study, we confirmed both positive neuroimaging findings and epilepsy surgery were associated with better seizure outcomes. Practically, patients with either neuroimaging-confirmed epileptic foci and/or drug-resistant seizures prefer to pursue epilepsy surgery

TABLE 2 Baseline information of the 27 patients who underwent epilepsy surgery.

	GATOR1	*DEPDC5*	*NPRL2*	*NPRL3*
Epilepsy surgery	27	18	4	5
Gender (male: female)	17:10	11:7	4:0	2:3
Age at seizure onset (m)	3 (0.1-72) (81.5%, <12 m)	3.5 (0.1-72) (77.8%, <12 m)	0.85 (0.17-2) (100%, <12 m)	4 (0.17-18) (80%, <12 m)
Seizure types				
Focal	24/27	16/18	4/4	4/5
Spasms	11/27	9/18	1/4	1/5
>2 seizure types	14/27	10/18	2/4	2/5
Epilepsy types				
IESS	11/27	9/18	1/4	1/5
West syndrome	5/27	5/18	0/4	0/5
Family history of epilepsy	6/27	6/18	0/4	0/5
Inheritance				
De novo	3/27	3/18	0/4	0/5
Inherited	24/27	15/18	4/4	5/5
Neuroimaging				
Positive MRI findings	20/27	12/18	4/4	4/5
Positive MRI + FDG-PET findings	27/27	18/18	4/4	5/5
Duration of epilepsy prior to surgery (m)	34 (8-105)	48.5 (10-105)	10.5 (8-14)	31(26-44)
ASMs	4 (3-8)	4 (3-8)	4.5 (4-6)	4 (3-7)
Surgery type				
Lobectomy	22/27	15/18	4/4	3/5
Hemispherotomy	4/27	3/18	0/4	1/5
Radiofrequency thermocoagulation	1/27	0/18	0/4	1/5
Surgery outcome				
Engel score I	25/27	17/18	4/4	4/5
Engel score II	2/27	1/18	0/4	1/5
Length of follow-up (m)	24 (12-60)	24 (12-60)	24 (12-36)	24 (24-36)
Histopathological diagnosis				
FCD Ia	3/27	1/18	0/4	2/5
FCD Ib	2/27	1/18	0/4	1/5
FCD IIa	15/27	11/18	3/4	1/5
mMCD	7/27	5/18	1/4	1/5

Abbreviations: ASMs, antiseizure medications; FCD, focal cortical dysplasia; FDG-PET, F-18-fluorodeoxyglucose positron emission tomography; IESS, infantile epileptic spasm syndrome; mMCD—mild malformations of cortical development; MRI, magnetic resonance imaging; IESS encompasses West syndrome.

treatment. And we compared the prognosis differences among patients with positive neurogaming findings. Among them, patients with seizure-free had more percentage of undergoing epilepsy surgery. Therefore, the most important prognostic factor for GATOR1-related epilepsy outcome is epilepsy surgery treatment. Favorable surgical outcomes in patients with GATOR1 variants have also been reported in recent studies, with 8/16 and 6/10 achieving favorable seizure outcomes in a systematic review[21] and multicenter study, respectively.[6] Our cohort included the most surgical patients so far; seizure freedom (Engel I) was achieved in 92.6% of patients, and a good surgical outcome (Engel I–II) was achieved in 100%. This finding may reflect the pathogenesis of GATOR1-related epilepsy. Germline GATOR1 loss-of-function variants result in overactivation of the mTOR

TABLE 3 Univariate comparison of prognostic factors.

	Seizure outcomes		
	Seizure-free ($n=29$)	**Seizure-ongoing ($n=21$)**	***P*-value**
Gender (male:female)	17:12	14:7	0.56
Age at seizure onset (m)	4 (0-87)	10 (0-124)	0.159
<12 m	21/29 (72.4%)	11/21 (52.4%)	0.15
Seizure types			
Focal	26/29 (89.7%)	17/21 (81.0%)	0.43
Spasms	11/29 (37.9%)	9/21 (42.9%)	0.73
>2 seizure types	14/29 (48.3%)	7/21 (33.3%)	0.29
Epilepsy types			
IESS	11/29 (37.9%)	8/21 38.1%)	0.99
West syndrome	5/29 (17.2%)	1/21 (4.8%)	0.38
Family history of epilepsy	6/29 (20.7%)	6/21 (28.6%)	0.52
Gene			0.16
DEPDC5	21/29 (72.4%)	16/21 (76.2%)	
NPRL2	4/29 (13.8%)	0/21 (0%)	
NPRL3	4/29 (13.8%)	5/21 (23.8%)	
Inheritance			0.69
De novo	3/29 (10.3%)	3/21 (14.3%)	
Inherited	26/29 (89.7%)	18/21 (85.7%)	
Novel classification framework			0.18
P	23/29 (79.3%)	13/21 (61.9%)	
LP	6/29 (20.7%)	8/21 (38.1%)	
Neuroimaging			**<0.001***
Positive findings	27/29 (93.1%)	11/21 (52.4%)	
ASMs	4 (1-8)	3 (2-8)	0.065
Therapy			**<0.001***
ASMs alone	4/29 (13.8%)	19/21 (90.5%)	
Epilepsy surgery	25/29 (86.2%)	2/21 (9.5%)	

Abbreviations: ASMs, antiseizure medications; IESS, infantile epileptic spasm syndrome; LP, likely pathogenic; P, pathogenic.

*P-value <0.05; IESS encompasses West syndrome.

pathway,[19] which in turn leads to structural epileptogenic malformations, such as FCD.[22,23] Theoretically, epilepsy surgery could be a curative etiological treatment for epileptogenic malformations. The previously reported higher surgical success rate of patients with other mTOR pathway gene mutations than patients with the non-mTOR gene mutations supported this hypothesis. Patients with other germline mTOR pathway genes, such as *PIK3CA*, *AKT3*, *PTEN*, and *mTOR*, have been reported to undergo epilepsy surgery, and 16/19 of them achieved seizure-free after surgery.[12] Therefore, if drug-resistant epilepsy with GATOR1 variants shows epilepsy-related neuroimaging findings, surgical treatment should be considered more aggressively.

Seven patients undergoing epilepsy surgery showed negative MRI findings. In the presurgical evaluation, surgeries were considered based on the stereotypical focal seizure, consistent abnormal EEG localization, results from functional imaging, and sEEG findings. Herein, seven patients had epileptic malformations on FDG-PET. Besides, sEEG was performed in four patients to confirm epileptic foci. Among patients with genetic epilepsy, MRI-positive patients tended to achieve higher seizure-free rates than MRI-negative patients.[12,24] In this study, all seven MRI-negative patients achieved seizure freedom. Findings from histological examinations confirmed FCD in five MRI–negative patients and mMCD in 2. These findings suggest that MRI negativity should

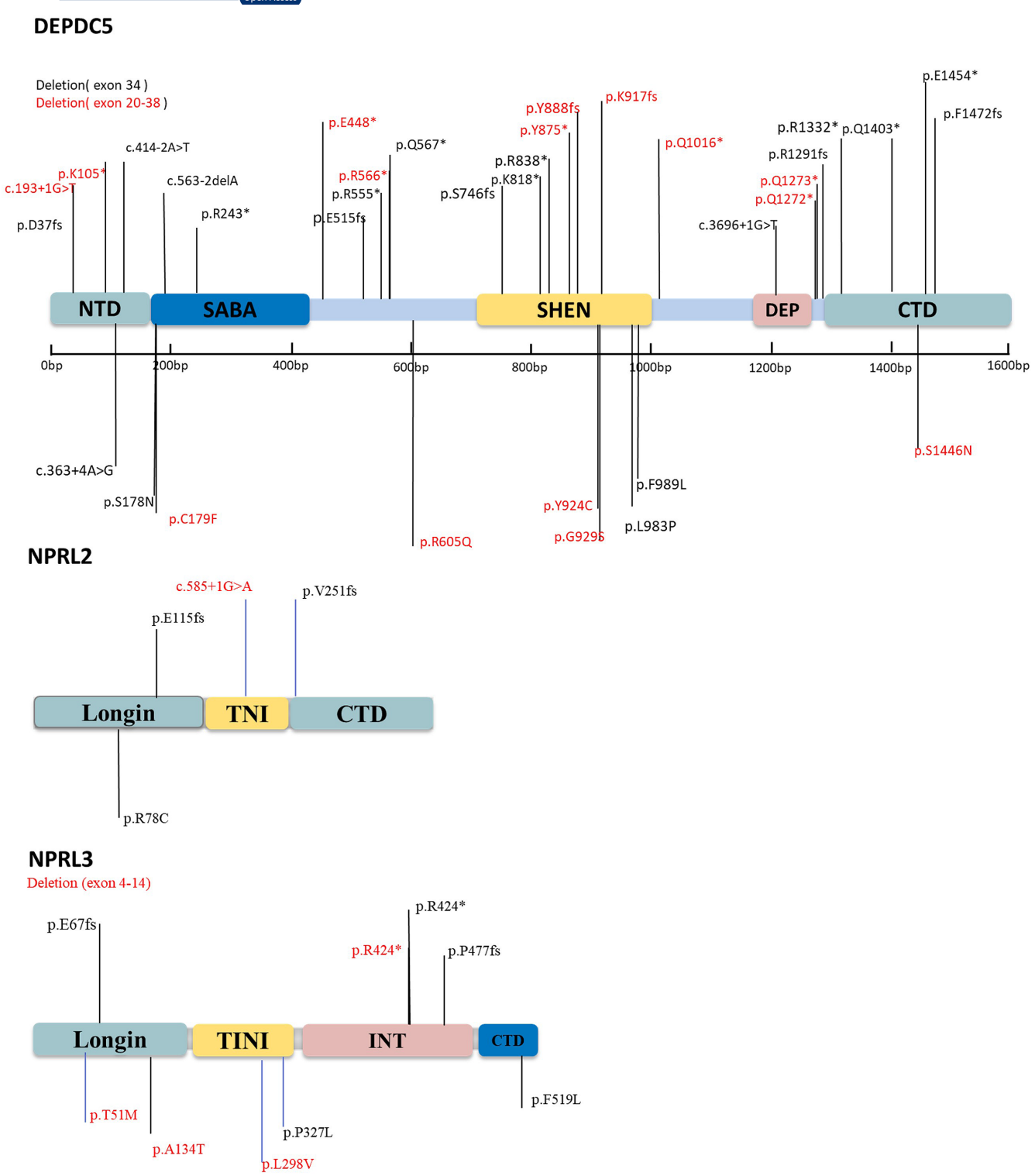

FIGURE 1 Schematic representation of the locations of 50 GATOR1 variants. In the upper part, variants are indicated as loss of function (LoF); in the bottom part, the missense variants and other splice-region variants are shown. Variants with poor prognosis are indicated in red.

not be an absolute contraindication for GATOR1-related epilepsy and that more presurgical investigations need to be conducted.

Multiple types of MCD have been reported in individuals with GATOR1 related epilepsy, including FCD, polymicrogyria, hemimegalencephaly, and bottom-of-sulcus dysplasia.[25] As previously reported, FCD was the most common histopathological type, which was observed in 74.1% (20/27) of the surgery patients in our cohort. The histological examinations of the other seven patients showed the

existence of blurred gray–white matter boundaries or immature neurons but with normal cortical lamination, lack of alterations in radial or tangential structure, and lack of dysmorphic neurons or balloon cells. These findings could not meet the characteristic of any subtype of FCD. And according to the ILAE guidelines,[26] they were classified as mMCD. The fuzzy findings may be due to technical reasons: the limitations of the sampling sites or specimen size.

ACMG guidelines were adapted to interpret the pathogenicity of variants identified in genes that cause Mendelian disorders. However, owing to the lack of function investigation and segregation evidence, some rare GATOR1 variants were classified as VUS according to the ACMG framework. Based on gnomAD frequency and mendelian clinically applicable pathogenicity (M-CAP) or human splice finder v3.1 (HSF) for in silico prediction, Baldassari et al.[6] proposed a novel classification framework specifically adapted to GATOR1 variants to help clinicians interpret the pathogenicity of GATOR1 variants. By applying this classification framework, they reclassified the 12 missense/splice–region variants as LP variants. And according to the novo classification framework, we reclassified 13 missense variants and one noncanonical splice-site variant as LP, one frameshift indel as P. Besides, we found that patients with reclassified P/LP variants had a similar phenotype and seizure outcome as those with P/LP variants interpreted by ACMG, indicating this novo classification framework may be more suitable for GATOR1 variants.

Most GATOR1 variants were inherited from asymptomatic parents, only four variants were from symptomatic parents and six variants were de novo. Previous research had the same conclusion. They found that GATOR1 variants were dominantly inherited from an asymptomatic parent in 64% (29/45) of the probands and only 4% (2/45) were de novo,[6] indicating more severe phenotype in the second generation. Multiple mechanisms may explain this phenotypic gradient. Firstly, patients with more severe phenotypes rarely have offspring, and they are always the probands. Secondly, the presence of second-hit somatic variants, which was the pathogenic mechanism of FCD, was another explanation. A biallelic second-hit somatic mutation was first found in a FCD patient with *DEPDC5* germline mutation.[22] Some other studies have demonstrated this hypothesis by finding more somatic variants in mTORopathies and second-hit animal models.[22,27] Unfortunately, we had not investigated somatic variants in brain samples after the surgery of our patients. Besides, the modifier gene may contribute to the clinical heterogeneity of GATOR1-related epilepsy. Iffland et al.[28] performed WES to search genetic modifiers in a large *NPRL3* c.349delG cohort. Though this study did not identify a specific genetic modifier, it provided an innovative way to understand the variability between generations.

Finally, we investigated the genotype–phenotype correlations of GATOR1-related epilepsy. In a previous study, Liu et al.[29] revealed that mutations closer to *DEPDC5*, *NPRL2*, and *NPRL3* binding sites may lead to a more severe phenotype, whereas Baldassari et al.[6] reported no significant genotype–phenotype correlation. We have not found the genotype–phenotype correlation here, suggesting the complex pathogenesis of this epilepsy.

This study has several limitations. First, these results were obtained from a retrospective cohort and a single institution, which carries a certain risk of selection bias, such as more early-onset epilepsy and drug-resistant epilepsy. Second, some probands underwent epilepsy gene panel testing and some VCFs were unavailable, making it difficult to rule out other possible causative mutations in some patients.

In conclusion, this retrospective study showed that epilepsy related to GATOR1 variants had high possibility to be drug-resistant epilepsy and to have positive neuroimaging finding. Epilepsy surgery is the only favorable factor for better seizure prognosis in this kind epilepsy. For MRI-negative patients, a further combination of FDG-PET and sEEG may increase the chance of finding the brain epileptic focus.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose. The authors confirm that they have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

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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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