Received: 9 May 2023 | Accepted: 20 October 2023

DOI: 10.1002/epi4.12856

Epilepsia Open® Open Access

CRITICAL REVIEW

Phenotypic and genotypic characterization of *NPRL3*-related epilepsy: Two case reports and literature review

Dongling Yang[1,2] | **Jinqiu Wang**[2] | **Zailong Qin**[3,4] | **Juntan Feng**[2] | **Chengyun Mao**[2] | **Yuyi Chen**[2] | **Xuelin Huang**[2] | **Yiyan Ruan**[2,3]

[1]Ruikang Clinical Medical College, Guangxi University of Chinese Medicine, Nanning, China

[2]Department of Pediatric Neurology, Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Guangxi Clinical Research Center for Pediatric Diseases, Nanning, China

[3]Guangxi Key Laboratory of Precision Medicine for Genetic Diseases, Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Nanning, China

[4]Genetic and Metabolic Central Laboratory, Guangxi Birth Defects Research and Prevention Institute, Nanning, China

Correspondence
Yiyan Ruan, Department of Pediatric Neurology, Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Guangxi Clinical Research Center for Pediatric Diseases, No. 59, Xiangzhu Avenue, Nanning, Guangxi 530012, China.
Email: 641039085@qq.com

Funding information
Yiyan Ruan, Grant/Award Number: Guike AB17195011;Guike AD22035121;GXWCH ZDKF-2022-

Abstract

Nitrogen permease regulator-like 3 (*NPRL3*) has been reported to play a role in seizure onset. The principal manifestation of *NPRL3*-related epilepsy is a range of epilepsy-associated syndromes, such as familial focal epilepsy with variable foci (FFEVF), sleep-related hypermotor epilepsy (SHE), and temporal lobe epilepsy (TLE). The association between phenotype and genotype of *NPRL3* mutations remains inadequately described. This study aimed to explore the phenotypic and genotypic spectra of *NPRL3*-related epilepsy. We reported two novel *NPRL3* variants in two unrelated epilepsy cases, including a nonsense (c.1174C > T, p.Gln392*) and a missense variant (c.1322C > T, p.Thr441Met). Following a review of the literature, a total of 116 cases of *NPRL3*-related epilepsy were assessed, mostly with nonsense and frameshift mutations. Our findings suggest that patients harboring various *NPRL3* variants exhibit variable clinical manifestations. In addition, it may be worthwhile to consider the existence of *NPRL3* mutations in epilepsy patients with a family history. This study provides useful information for the treatment and prognosis by expanding the phenotypic and genotypic spectrum of *NPRL3*-related epilepsy.

Plain Language Summary

This study expands the phenotypic and genotypic spectra of NPRL3-related epilepsy by reporting two cases with different novel variants. Following a review of the literature, it was observed that patients harboring various NPRL3 variants exhibited a variability of clinical manifestations. Also, patients carrying nonsense mutations are frequently prone to drug resistance and other severe comorbidities such as developmental delay, but more cases need to be collected to confirm these findings.

KEYWORDS

focal epilepsy, genotype, mutation, *NPRL3*, phenotype

1 | INTRODUCTION

Genetic factors play a significant role in the occurrence and development of epilepsy. Recently, several epileptogenic genes encoding non-ion channel proteins have been reported, particularly the GTPase-activating protein activity toward Rags 1 (GATOR1) gene complex. The GATOR1 complex, which includes Egl-10 and domain-containing protein 5 (DEPDC5), nitrogen permease regulator-like 2 (NPRL2), and nitrogen permease regulator-like 3 (NPRL3), shows an inhibitory effect on the mechanistic target of rapamycin (mTOR) signaling pathway. It has been shown to regulate neurogenesis, synaptic transmission and plasticity.[1] *NPRL3* is a highly conserved and widely expressed gene associated with development, and it is located on chromosome 16p13.3 and encodes a protein of 569 amino acids.[2] Mutation in *NPRL3* can lead to the loss of function of the GATOR1 complex, thereby excessively activating the mTOR signaling pathway, and this can affect the morphology, structure and function of brain cells.[3]

The common phenotypes of *NPRL3*-related epilepsy are familial and sporadic focal epilepsy with or without focal cortical dysplasia (FCD), especially familial focal epilepsy with variable foci (FFEVF).[4,5] Notably, there are remarkable differences in the clinical manifestations of focal epilepsy which usually occur in diverse cortical areas in various family members. Furthermore, several epilepsy syndromes, such as sleep-related hypermotor epilepsy (SHE) and benign epilepsy in children with central temporal lobe spikes, are also associated with *NPRL3*.[3,6]

In this study, we expand the phenotypic spectrum of NPRL3-related epilepsy by describing the clinical characteristics of two cases with different novel variants of NPRL3. In addition, we reviewed the reports related to similar conditions and summarized the associations between the phenotypes and genotypes in NPRL3-related epilepsy. This study attempts to customize the treatment strategies and evaluation of the prognosis of this condition.

Key points

- Patients carrying different *NPRL3* variants exhibit a variability of clinical manifestations.
- Patients harboring nonsense mutations tend to suffer from drug resistance and other severe comorbidities.
- The penetrance of *NPRL3*-related epilepsy is incomplete.
- The *NPRL3* variant should be considered in patients with a family history of epilepsy.

2 | METHODS

2.1 | Case presentation

2.1.1 | Case 1

A girl, aged 2 years and 5 months, had an onset of unprovoked seizures 10 days after birth, which mainly manifested as tonic seizures, epileptic spasms and focal seizures. The seizures occurred mostly during wakefulness and starvation and lasted for 20–40 s at a frequency of more than 20 times per day. The seizures were difficult to control using a ketogenic diet and adrenocorticotropic hormone (ACTH), as well as a range of anti-seizure medications (ASMs), including topiramate, levetiracetam, valproate, clonazepam, oxcarbazepine, lacosamide and perampanel. The patient was born at term and delivered by cesarean section with normal performance. She developed obvious developmental delay at 4 months of age whereby she failed to raise her head. She also had difficulties tracking moving objects and responding to sounds. Notably, her grandmother and uncle had a suspected history of epilepsy while her father was healthy without any known history of serious illness.

Metabolic screening, cerebrospinal fluid tests and routine biochemical examinations were unremarkable. However, brain magnetic resonance imaging (MRI) revealed abnormal signals around the posterior horn of the left lateral ventricle and a widened left frontotemporal sulcus. A widened bilateral frontotemporal extracerebral space was observed 1 month later (Figure S1). Video electroencephalogram (VEEG) demonstrated diffuse slow wave activity with a prominent left posterior head, slow physiological waves in the left frontal, central, parietal, and meso-posterior temporal regions, and spike-waves in the left central and parietal regions during sleep (Figure S2). A heterozygous NM_001077350.3: c.1174C > T (p.Gln392*) mutation in *NPRL3* was identified in the proband and her father by whole-exome sequencing (WES). She was ultimately diagnosed with FFEVF. The patient had been seizure-free by hemispherectomy at 1 year and 2 months of age, and yet she has been undergoing rehabilitation training due to cerebral palsy manifested on her right side.

2.1.2 | Case 2

A 6-year-old girl had repeated seizures for more than 5 years. The patient was born without asphyxia or hypoxia and had no family history of epilepsy. Focal seizures and epileptic spasms occurred with no inducing factors at

10 months of age, which mainly manifested as bilateral gaze, teeth biting, right limb convulsions, or head nodding with flexion of upper limbs. The seizures occurred mostly before sleep and lasted for 20–60 s at a frequency of more than 10–30 times per day. Her cerebral MRI appeared normal. Conversely, VEEG revealed generalized slow and fast wave activities, frequent either single or serial spasms during sleep as well as a secondarily generalized seizure (Figure S3). WES demonstrated the proband and her mother carried a heterozygous variant of *NPRL3* (NM_001077350.3: c.1322C > T, p.Thr441Met). Other examinations were unremarkable except for severe developmental delay. Significantly, her mother had a suspected intellectual disorder, and her biological older brother and sister also have been suspected of having a developmental delay. The final diagnosis of the patient was FFEVF.

Seizures remarkably decreased after ASMs treatment (topiramate, sodium valproate, nitrazepam and methylprednisolone), whereas levetiracetam was ineffective. The girl had been seizure-free for 8 months when treated with a combination of valproate, topiramate and nitrazepam. Nonetheless, the irregularity of ASMs intake gave rise to the frequent occurrence of seizures, which lasted for 40–60 s at a frequency of more than 20 times per day. The patient also presented with focal to bilateral tonic–clonic seizures (FBTCS). Multifocal and generalized spike, polyspike, spike slow and slow-wave discharges were observed during the VEEG re-examination (Figure S4). After ACTH pulse therapy, the patient who continues to take valproate, topiramate and nitrazepam, experienced only a few seconds of limb tremors 2–3 times a day.

2.2 | Novel mutation in NPRL3 gene

Peripheral venous blood was collected from two patients and their parents for WES. Two novel mutations in *NPRL3* gene (NM_001077350.3) were detected, c.1174C > T (p.Gln392*) and c.1322C > T (p.Thr441Met) (Figure 1). The results of Sanger sequencing validated that the proband and the father carried the same variant in Case 1. And the mother was a mild-type, that is, did not carry the NPRL3 gene variant, whereas in Case 2, the proband and the mother carried the same variant with a mild-type father. The variant c.1174C > T is a nonsense mutation found in Case 1, in which sequence change creates a premature translational stop signal (p.Gln392*) in the NPRL3 gene. It is predicted to affect protein products and result in loss of function mutation (LoF). This variant had neither been previously found in the Asian Population database of 1000 Genomes Project nor recorded in the ExAC and gnomAD databases. ClinVar contains an entry for this variant (Variation ID: 1072659). Based on the standards and guidelines for the American College of Medical Genetics and Genomics (ACMG), this variant has been classified as Pathogenic.[7]

The variant c.1322C > T is a missense mutation found in Case 2, in which sequence change replaces neutral and non-polar threonine with methionine at codon 441 of the NPRL3 protein (p.Thr441Met). This variant has been recorded in the dbSNP database (rs776673140) and ClinVar (Variation ID: 1500544). The functional significance of the variant c.1322C > T is currently obscure. The present evidence is inconclusive as to role of this variant in disease. According to the ACMG standards and guidelines, it has been classified as a Variant of Uncertain Significance (VUS).[7]

2.3 | Literature review

A systematic literature search was performed in PubMed (accessed February 2023). The research strategy was as follows: "NPRL3" AND "epilepsy". Data from all eligible studies were analyzed and discussed by two reviewers. A list of reported *NPRL3* variants-related epilepsy is provided in Table S1.

3 | DISCUSSION

The mTOR signaling plays an important role in neurogenesis, synaptic transmission and plasticity during embryonic brain development. *NPRL3* encodes a subunit of the GATOR1 complex, which is an upstream regulatory element of the mTOR pathway.[8] The GATOR1 complex negatively modulates the activity of the mTOR pathway in response to extracellular amino acid level changes.[8,9] GATOR1 dysfunction caused by *NPRL3* variants can ramp up the activity of the mTOR signaling pathway, thereby affecting brain development and function.[8] Enlarged neurons and mTOR pathway activation were observed in the brain tissue samples obtained from individuals with *NPRL3*-related FCD.[3] Iffland et al.[10] found mTOR-dependent abnormalities in the cell size and subcellular translocation of mTOR in *NPRl3*-knockout mouse neuronal cell lines. The first animal model of *NPRL3*-related epilepsy was established by Ishida et al. deleted *NPRl3* from the dorsal telencephalon in mice. This recapitulated spontaneous seizures and led to dysmorphically enlarged neuronal cells with increased mTORC1 signaling.[11] Overall, *NPRL3* variants might contribute to seizures by interfering with the mTOR signaling pathway.

To the best of our knowledge (up to February 2023), 114 patients with *NPRL3*-related epilepsy have been reported.[3–6,12–27] Together with our reported cases, there

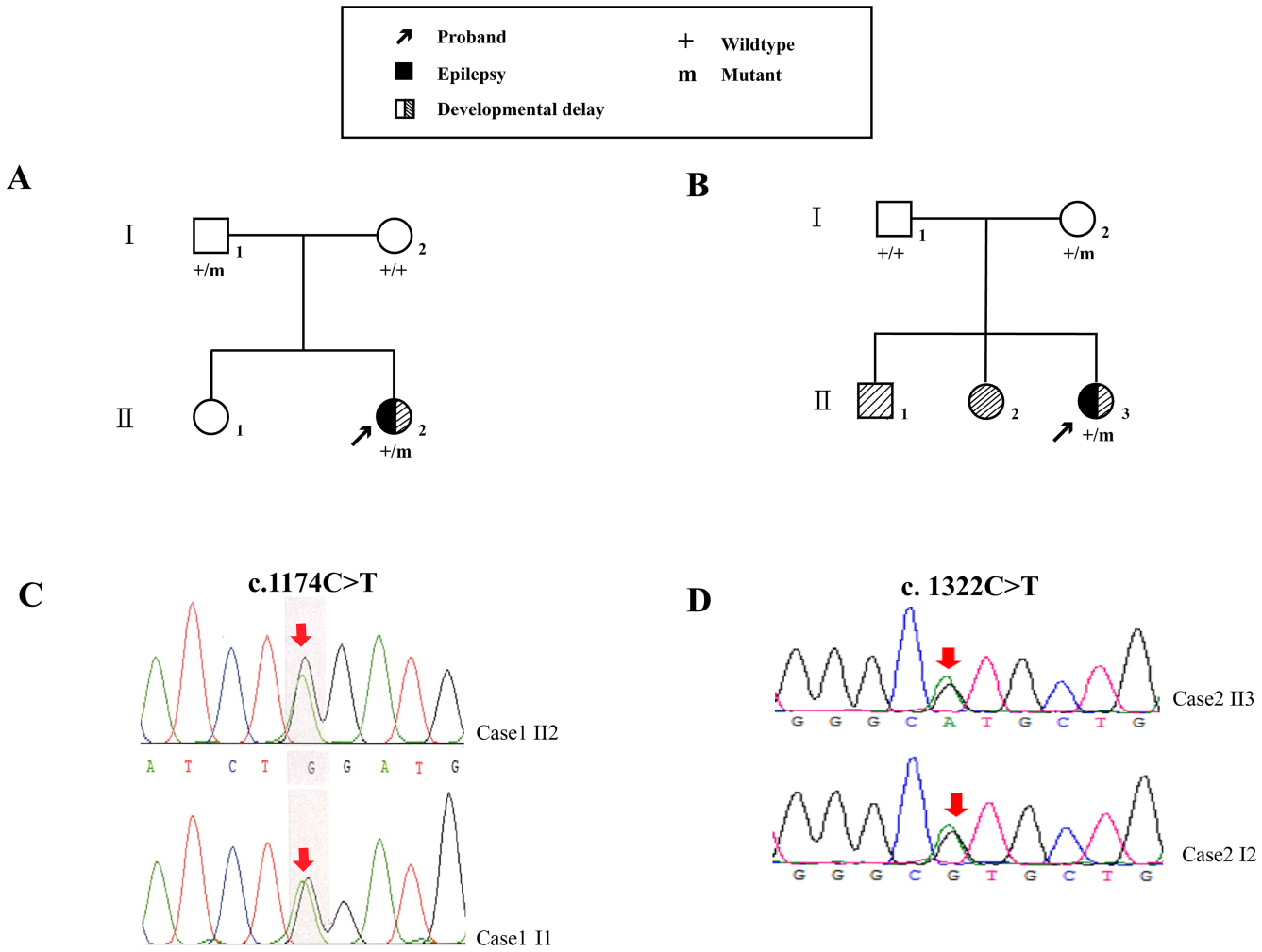

FIGURE 1 The pedigrees and Sanger sequencing results of the two cases with *NPRL3* variants. (A, C and B, D refer to Cases 1 and 2, respectively).

were 116 patients of which 42 and 31 were male and female, respectively, and 43 cases were of unknown gender (Table 1, Table S1). The age of onset was obtained for 65 cases, and this suggested a wide onset age range for *NPRL3*-related epilepsy, ranging from the neonatal period to adulthood. Focal seizure was the most common type of seizure in NPRL3-related epilepsy. Epileptic syndromes included FFEVF (24 cases) and SHE (12 cases). Apart from unclassified epilepsy (UE) and unclassified focal epilepsy (FE), epileptic phenotype included frontal lobe epilepsy (FLE) (9 cases), temporal lobe epilepsy (TLE) (8 cases), tonic–clonic seizure (TCS) (3 cases) and febrile seizures/febrile seizures plus (FS/FS+) (2 cases). Neonatal seizures (1 case), frontotemporal epilepsy (1 case) and epilepsy with auditory features (1 case) were also observed (Table 1, Table S1). In addition, multiple seizure types were identified in 3 cases, one of which first presented with FS at 5 months of age and then TCS at 12 years old. In our reported cases, one patient suffered multiple types of seizure (including tonic seizure, epileptic spasm and focal seizure) 10 days after birth, and the other had epileptic spasms, focal seizures which were followed by focal to generalized tonic–clonic seizures.

It should be noted that there were no structural intracranial lesions observed in most *NPRL3*-related epilepsy cases. Brain MRI results were acquired from 68 cases, and this included 26 cases with abnormal MRI. A total of 23 patients with abnormal MRI were diagnosed with malformation of cortical development (MCD), 18 of whom presented with FCD, 3 with hemimegalencephaly (HME), 1 with periventricular heterotopia (PH) and 1 with polymicrogyria (PMG) (Table 1, Table S1). FCD IIa (7 cases) and FCD IIb (1 case) were confirmed by postoperative histology examinations in 8 patients with FCD who underwent surgery (Table S1). In our reported cases, Case 1 was considered to have a high likelihood of FCD after MRI consultations at an external hospital. In addition, there was one case each of hippocampal sclerosis, brain atrophy and cortical thickening of the frontal gyrus observed from the reported cases. A "two-hit" hypothesis has been considered

TABLE 1 Summary of the genotypic and phenotypic characteristics of *NPRL3*-related epilepsy.

Phenotypic characteristics	Cases with *NPRL3*-related epilepsy
Sex	31 F, 42 M (n = 73)
Age (Onset)	1 day ~51 years (n = 65)
FFEVF	24
SHE	12
TLE	8
FLE	9
TCS	3
FS/FS+	2
UE	12
MCD	22 (17 FCD, 3 HME, 1 PH, 1 PMG)
Brain MRI	42 Normal, 26 Abnormal (n = 68)
EEG	10 Normal, 63 Abnormal (n = 73)
Development delay	7
Drug resistance	20
Familial/Sporadic	98 Familial/9 Sporadic
Inheritance	98 Inherited/7 *de novo*
Genotypic characteristics	**Cases with *NPRL3* variants (n = 37)**
Variant class	
Nonsense	12
Frameshift	10
Missense	8
Exonic deletion	5
Splicing	2
Novel classification	
Pathogentic	24
VUS	10
Likely pathogentic	3

Abbreviations: EEG, electroencephalogram; F, female; FCD, focal cortical dysplasia; FFEVF, familial focal epilepsy with variable foci; FLE, frontal lobe epilepsy; FS, febrile seizures; FS+, febrile seizuresplus; HME, hemimegalencephaly; LOF, loss of function; M, male; MRI, magnetic resonance imaging; PH, periventricular heterotopia; PMG, Polymicrogyria; SHE, sleep-related hypermotor epilepsy; TCS, tonic–clonic seizures; TLE, temporal lobe epilepsy; UE, unclassified epilepsy; VUS, variants of uncertain significance.

as a possible mechanism for GATOR1 complex-related epilepsy either with or without MCD. This would involve a germline variation in the GATOR1 complex gene, which is followed by cellular variation in the other allele that is influenced by environmental and modifier genes, resulting in inactivation of both alleles in the focal area and then cortical malformation.[27] Germline and somatic mutations causing FCD were identified in the focal tissues of patients carrying the *DEPDC5* variants.[28,29] A maternally inherited pathogenic germline variant in the *NPRL3* gene from two brothers with FCD was reported by Bennett et al.[21] However, Baulac et al. reported a case of autosomal dominant sleep-related hypermotor epilepsy (ADSHE) with both germline and somatic variants of the *DEPDC5* gene without significant imaging abnormalities.[30] Hence, the pathogenesis of MCD needs to be further investigated.

It was reported that around 60% of GATOR1-related epilepsy patients had either cognitive impairment or psychosomatic comorbidities.[31] That was especially the situation in patients with early onset, poor seizure control and epileptic spasms.[31] Moreover, GATOR1-related epilepsy with attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) were also reported.[31] In a literature review, several patients presented with development delay (7 cases), cognitive impairment (2 cases) and oppositional disorder (1 case) after the onset of seizures (Table 1, Table S1). The other patients suffered FLE combined with intellectual disability (ID) and ASD. In addition, sudden unexpected death in epilepsy (SUDEP) was suspected in the families of 3 cases, although whether the dead family members carried the same variants could not be verified. Baldassari et al. reported that SUDEP occurred in 17 out of 183 patients who had GATOR1 complex gene mutations, which indicated the incidence of SUDEP in GATOR1-related epilepsy relatively was high (9.3%).[14] Currently, though the pathophysiological mechanism of SUDEP is still unknown, early onset, sleep-related onset and drug resistance are potential risk factors.

Previous studies have suggested that the most common GATOR1 complex gene variants are loss-of-function (LoF) variants, which account for 67%, and these include nonsense, frameshift and splicing variants, followed by missense variants.[14] Combined with the reported cases, a total of 37 *NPRL3* variants were detected, with nonsense variants being the most common (12 cases), followed by frameshift variants (10 cases), missense variants (8 cases), exonic deletion (5 cases) and splicing variants (2 cases) (Table 1, Figure 2). *NPRL3* LOF mutations inhibit the negative regulation of the GATOR1 complex on mTORC1, which leads to hyper-activation of the mTOR signaling pathway. Li et al. found that transcription and expression of *NPRL3* in Chinese FFEVF-associated families were severely affected. This results in hyper-activation of the mTOR signaling pathway and coincides with the observations of Canavati et al., who suggested that the truncated variants of *NPRL3* can significantly reduce the transcription levels.[15,20] Nevertheless, the pathogenic mechanism of missense variants needs to be further explored. Currently, haploid insufficiency is the main pathogenic mechanism for the GATOR1 complex gene variation.[4] All the reported *NPRL3*-related epilepsy were heterozygous mutations.

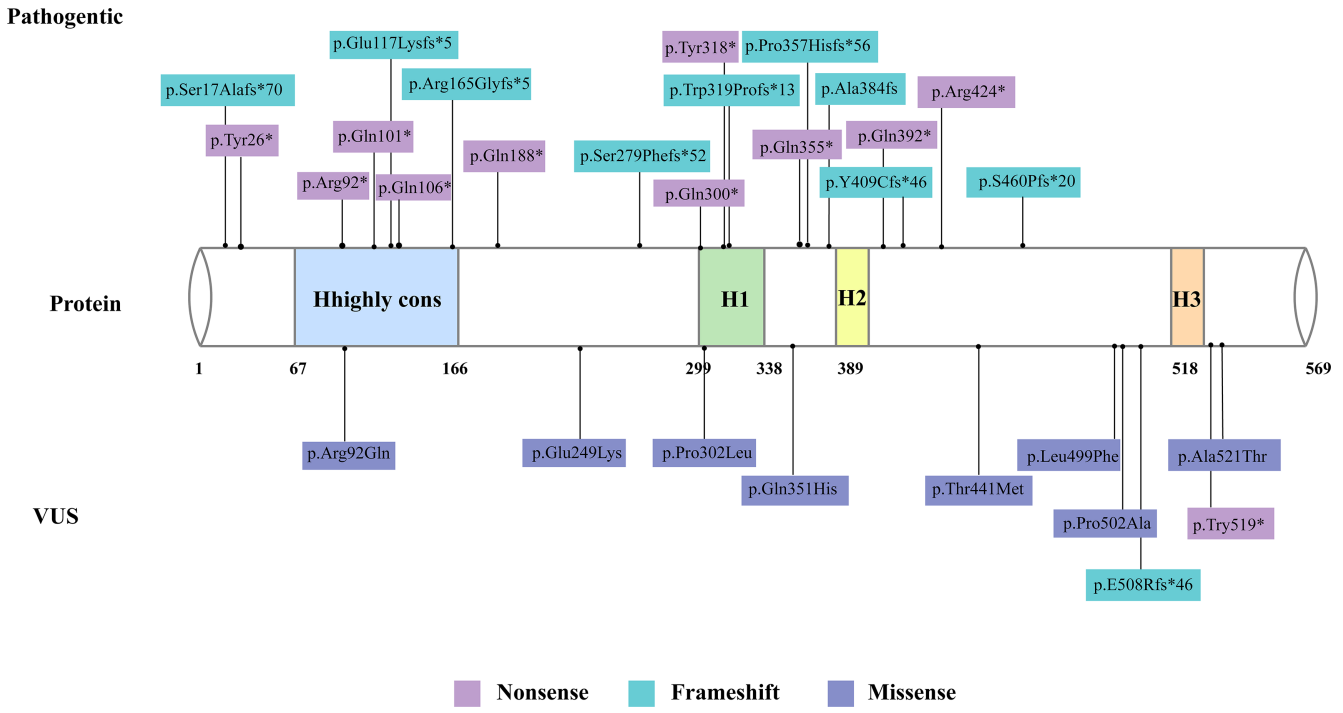

FIGURE 2 Diagrammatic representation of the NPRL3 protein. The highly conserved region at the N-terminus is highlighted in blue, the positions of the three helix-turn-helix domains H1, H2, and H3 are highlighted in green, yellow, and orange–pink, respectively.

The GATOR1 complex gene mutation is autosomal dominant with incomplete penetrance. It was reported that over 90% of patients carried inherited variants.[14] Different epileptic phenotypes occur in different patients with the same variant seen in the same family, ranging from no symptoms to SUDEP. Among the 105 cases with *NPRL3*-related epilepsy reviewed here, 98 carried inherited variants and only seven with de novo variants (Table 1, Table S1). 13 of the 20 *NPRL3*-related families were reported to manifest incomplete epilepsy penetrance. In a four-generation family with six cases of nocturnal frontal lobe epilepsy, individuals carrying the *NPRL3* heterozygous mutations were found to present seizure-free, with an overall penetration of at most 50%.[6] Li et al.[20] reported a six-generation Chinese family with 8 FFEVF patients carrying the *NPRL3* mutation and the overall penetrance was 50% or less. An *NPRL3*-related epilepsy penetrance rate of 28% was reported in Iffland et al.[10] In our two cases, two probands' relatives were observed to carry *NPRL3* variants without epileptic phenotypes, which further indicated that the penetrance of *NPRL3*-related epilepsy is incomplete.

Our study found that some specific mutations may lead to more severe clinical manifestations. Patients carrying nonsense (7/20), exonic deletion (4/20) and missense mutations (4/20) were more susceptible to drug resistance (Table 1, Table S1). A total of 15 out of 22 cases suffering MCD carried frameshift mutations. Other severe comorbidities such as developmental delay, ID, ASD, and even SUDEP were more likely to be observed in patients carrying nonsense and missense mutations (Table S1). Conversely, splicing mutations were the less common variants (2/116) of all cases.

Phenotypic and genotypic characteristics can provide a scientific basis for treatments of *NPRL3*-related epilepsy. Resistance to antiepileptic drug therapy has been reported in nearly 50% of patients with *NPRL3*-related epilepsy.[12,14,18] A majority of patients with definite epileptogenic focus were either seizure-free or had significant improvements after surgical resection. For patients with no structural lesions and nonsurgical indications, the mTOR inhibitor, rapamycin, maybe a new treatment option. Previous publications reported that mTOR inhibitors can achieve long-term seizure reduction and overall improvements in the disease.[32] However, a case of *NPRL3*-related epilepsy was reported in which seizures were effectively controlled with rapamycin but ultimately failed due to various side effects.[33] Therefore, mTOR inhibitors need to be researched more thoroughly before being used routinely in these types of cases. Additionally, GATOR1 complex agonists, which are more selective than mTOR inhibitors, may be a promising new therapeutic target.[28]

There were certain limitations in this study. Only the patients and their parents participated in genetic testing in these two *NPRL3*-related epilepsy cases with novel variants. However, the whole pedigree analysis was not performed and therefore we failed to explore the relevance between these mutations and clinical phenotypes.

Additional cases need to be collected, and their detailed phenotypic analysis conducted in order to evaluate whether identified the pathogenic variants contribute to the appearance of phenotype.

4 | CONCLUSION

Our study reported two novel *NPRL3* variants observed in two unrelated infants. These were due to a nonsense and a missense variants, and both cases further evidenced the involvement of *NPRL3* in epileptogenesis. We observed that patients harboring various *NPRL3* variants exhibited a variability of clinical manifestations. Also, patients carrying nonsense mutations are frequently prone to drug resistance and other severe comorbidities such as developmental delay. Our study expands the phenotypic and genotypic spectra of *NPRL3*-related epilepsy. In addition, the cases presented here will provide physicians with valuable information for the treatment and prognostic evaluation of new patients with similar conditions.

AUTHOR CONTRIBUTIONS

Dongling Yang was involved in the design of the study, analyses of data, and writing of the manuscript. Jinqiu Wang was involved in data acquisition, data interpretation, and data curation. Zailong Qin was involved in the interpretation of data and manuscript writing. Juntan Feng was involved in data interpretation and data curation. Chengyun Mao was involved in the acquisition of data and curation of data. Yuyi Chen was involved in the analyses of data. Xuelin Huang was involved in data acquisition. Yiyan Ruan was involved in the writing of the manuscript and funding acquisition.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Dev Sooranna, Imperial College London, for editing the manuscript.

FUNDING INFORMATION

This work was supported by financial grants from the Guangxi Science and Technology Program Project (Guike AB17195011, Guike AD22035121); the Guangxi Key Laboratory Open Project (GXWCH ZDKF-2022-16); and the Guangxi Key Subject of Medical and Health Project.

CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

Data to support the findings of this study are included in the article and supplementary materials. Additional data may be available upon reasonable request.

ETHICS STATEMENT

Written informed consent was obtained from the parents or legal representatives of the involved patients. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with these guidelines.

ORCID

Dongling Yang https://orcid.org/0000-0001-8107-1625
Yiyan Ruan https://orcid.org/0000-0003-0857-196X

REFERENCES

1. Shen K, Huang RK, Brignole EJ, Condon KJ, Valenstein ML, Chantranupong L, et al. Architecture of the human GATOR1 and GATOR1-rag GTPases complexes. Nature. 2018;556(7699):64–9. https://doi.org/10.1038/nature26158
2. Levine TP, Daniels RD, Wong LH, Gatta AT, Gerondopoulos A, Barr FA. Discovery of new Longin and roadblock domains that form platforms for small GTPases in Ragulator and TRAPP-II[J]. Small GTPases. 2013;4(2):62–9. https://doi.org/10.4161/sgtp.24262
3. Ricos MG, Hodgson BL, Pippucci T, Saidin A, Ong YS, Heron SE, et al. Mutations in the mammalian target of rapamycin pathway regulators NPRL2 and NPRL3 cause focal epilepsy. Ann Neurol. 2016;79(1):120–31. https://doi.org/10.1002/ana.24547
4. Weckhuysen S, Marsan E, Lambrecq V, Marchal C, Morin-Brureau M, An-Gourfinkel I, et al. Involvement of GATOR complex genes in familial focal epilepsies and focal cortical dysplasia. Epilepsia. 2016;57(6):994–1003. https://doi.org/10.1111/epi.13391
5. Krenn M, Wagner M, Hotzy C, Graf E, Weber S, Brunet T, et al. Diagnostic exome sequencing in non-acquired focal epilepsies highlights a major role of GATOR1 complex genes. J Med Genet. 2020;57(9):624–33. https://doi.org/10.1136/jmedgenet-2019-106658
6. Korenke GC, Eggert M, Thiele H, Nürnberg P, Sander T, Steinlein OK. Nocturnal frontal lobe epilepsy caused by a mutation in the GATOR1 complex gene NPRL3. Epilepsia. 2016;57(3):e60–3. https://doi.org/10.1111/epi.13307
7. Richards S, Aziz N, Bale S, Bick D, das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24. https://doi.org/10.1038/gim.2015.30
8. Baldassari S, Licchetta L, Tinuper P, Bisulli F, Pippucci T. GATOR1 complex: the common genetic actor in focal epilepsies. J Med Genet. 2016;53(8):503–10. https://doi.org/10.1136/jmedgenet-2016-103883
9. Sancak Y, Sabatini DM. Rag proteins regulate amino-acid-induced mTORC1 signalling. Biochem Soc Trans. 2009;37(Pt 1):289–90. https://doi.org/10.1042/BST0370289
10. Iffland PH, Baybis M, Barnes AE, et al. DEPDC5 and NPRL3 modulate cell size, filopodial outgrowth, and localization of mTOR in neural progenitor cells and neurons. Neurobiol Dis. 2018;114:184–93. https://doi.org/10.1016/j.nbd.2018.02.013
11. Ishida S, Zhao D, Sawada Y, Hiraoka Y, Mashimo T, Tanaka K. Dorsal telencephalon-specific Nprl2-and Nprl3-knockout

mice: novel mouse models for GATORopathy. Hum Mol Genet. 2022;31(9):1519–30. https://doi.org/10.1093/hmg/ddab337
12. Sim JC, Scerri T, Fanjul-Fernandez M, et al. Familial cortical dysplasia caused by mutation in the mammalian target of rapamycin regulator NPRL3. Ann Neurol. 2016;79:132–7. https://doi.org/10.1002/ana.24502
13. Strauss KA, Gonzaga-Jauregui C, Brigatti KW, Williams KB, King AK, van Hout C, et al. Genomic diagnostics within a medically underserved population: efficacy and implications. Genet Med. 2018;20(1):31–41. https://doi.org/10.1038/gim.2017.76
14. Baldassari S, Picard F, Verbeek NE, van Kempen M, Brilstra EH, Lesca G, et al. The landscape of epilepsy-related GATOR1 variants. Genet Med. 2019;21(2):398–408. https://doi.org/10.1038/s41436-018-0060-2
15. Canavati C, Klein KM, Afawi Z, Pendziwiat M, Abu Rayyan A, Kamal L, et al. Inclusion of hemimegalencephaly into the phenotypic spectrum of NPRL3 pathogenic variants in familial focal epilepsy with variable foci. Epilepsia. 2019;60(6):e67–73. https://doi.org/10.1111/epi.15665
16. Deng J, Fang F, Wang XH, et al. Clinical and genetic characteristics of focal epilepsy in children caused by GATOR1 complex gene variation. Zhonghua Er Ke Za Zhi. 2019;57(10):780–5. Chinese. https://doi.org/10.3760/cma.j.issn.0578-1310.2019.10.010
17. Blümcke I, Coras R, Busch RM, Morita-Sherman M, Lal D, Prayson R, et al. Toward a better definition of focal cortical dysplasia: An iterative histopathological and genetic agreement trial. Epilepsia. 2021;62(6):1416–28. https://doi.org/10.1111/epi.16899
18. Abumurad S, Issa NP, Wu S, Rose S, Taylan Esengul Y, Nordli D, et al. Laser interstitial thermal therapy for NPRL3-related epilepsy with multiple seizure foci: a case report. Epilepsy Behav Rep. 2021;16:100459. https://doi.org/10.1016/j.ebr.2021.100459
19. Chandrasekar I, Tourney A, Loo K, Carmichael J, James K, Ellsworth KA, et al. Hemimegalencephaly and intractable seizures associated with the NPRL3 gene variant in a newborn: a case report. Am J Med Genet A. 2021;185(7):2126–30. https://doi.org/10.1002/ajmg.a.62185
20. Li Y, Zhao X, Wang S, Xu K, Zhao X, Huang S, et al. A novel loss-of-function mutation in the NPRL3 gene identified in Chinese familial focal epilepsy with variable foci. Front Genet. 2021;12:766354. https://doi.org/10.3389/fgene.2021.766354
21. Bennett MF, Hildebrand MS, Kayumi S, Corbett MA, Gupta S, Ye Z, et al. Evidence for a dual-pathway, 2-hit genetic model for focal cortical dysplasia and epilepsy. Neurol Genet. 2022;8(1):e652. https://doi.org/10.1212/NXG.0000000000000652
22. Iffland PH, Everett ME, Cobb-Pitstick KM, et al. NPRL3 loss alters neuronal morphology, mTOR localization, cortical lamination, and seizure threshold. Brain. 2022;145(11):3872–85. https://doi.org/10.1093/brain/awac044
23. Dunn PJ, Maher BH, Albury CL, Stuart S, Sutherland HG, Maksemous N, et al. Tiered analysis of whole-exome sequencing for epilepsy diagnosis. Mol Genet Genomics. 2020;295(3):751–63. https://doi.org/10.1007/s00438-020-01657-x
24. Hu J, Gao X, Chen L, Kan Y, du Z, Xin S, et al. Identification of two rare NPRL3 variants in two Chinese families with familial focal epilepsy with variable foci 3: NGS analysis with literature review. Front Genet. 2023;13:1054567. https://doi.org/10.3389/fgene.2022.1054567
25. Kovačević M, Janković M, Branković M, Milićević O, Novaković I, Sokić D, et al. Novel GATOR1 variants in focal epilepsy. Epilepsy Behav. 2023;141:109139. https://doi.org/10.1016/j.yebeh.2023.109139
26. Vasquez A, Miller KJ, Youssef PE, Selcen D, Patterson MC, Starnes K. A case report of hemimegalencephaly with super-refractory status epilepticus and brain atrophy associated with NPRL3 gene mutation. Seizure. 2023;S1059-1311(23):00051-1. https://doi.org/10.1016/j.seizure.2023.02.010
27. Baulac S. mTOR signalling pathway genes in focal epilepsies. Prog Brain Res. 2016;226:61–79. https://doi.org/10.1016/bs.pbr.2016.04.013
28. Myers KA, Scheffer IE. DEPDC5 as a potential therapeutic target for epilepsy. Expert Opin Ther Targets. 2017;21(6):1–600. https://doi.org/10.1080/14728222.2017.1316715
29. Ribierre T, Deleuze C, Bacq A, Baldassari S, Marsan E, Chipaux M, et al. Second-hit mosaic mutation in mTORC1 repressor DEPDC5 causes focal cortical dysplasia-associated epilepsy. J Clin Invest. 2018;128(6):2452–8. https://doi.org/10.1172/JCI99384
30. Baulac S, Ishida S, Marsan E, Miquel C, Biraben A, Nguyen DK, et al. Familial focal epilepsy with focal cortical dysplasia due to DEPDC5 mutations. Ann Neurol. 2015;77(4):675–83. https://doi.org/10.1002/ana.24368
31. Burger BJ, Rose S, Bennuri SC, Gill PS, Tippett ML, Delhey L, et al. Autistic siblings with novel mutations in two different genes: insight for genetic workups of autistic siblings and connection to mitochondrial dysfunction. Front Pediatr. 2017;5:219. https://doi.org/10.3389/fped.2017.00219
32. Hsieh LS, Wen JH, Claycomb K, Huang Y, Harrsch FA, Naegele JR, et al. Convulsive seizures from experimental focal cortical dysplasia occur independently of cell misplacement. Nat Commun. 2016;7:11753. https://doi.org/10.1038/ncomms11753
33. Vawter-Lee M, Franz DN, Fuller CE, et al. Clinical letter: a case report of targeted therapy with sirolimus for NPRL3 epilepsy. Seizure. 2019;73:43–5. https://doi.org/10.1016/j.seizure.2019.10.007

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: Yang D, Wang J, Qin Z, Feng J, Mao C, Chen Y, et al. Phenotypic and genotypic characterization of *NPRL3*-related epilepsy: Two case reports and literature review. Epilepsia Open. 2024;9:33–40. https://doi.org/10.1002/epi4.12856