■ Original article

Epileptic Disord 2021; 23 (4): 623-632

Epileptic Disorders

Evaluation of candidate genes in a Chinese cohort of atypical Rolandic epilepsy

Xiaoyue Hu[1,2], Jihong Tang[1], Ying Hua[2], Yanping Wang[2], Jing Huang[1]

[1]Department of Neurology, Children's Hospital of Soochow University, Suzhou, Jiangsu Province, China
[2]Department of Neurology, Wuxi Children's Hospital, Wuxi, Jiangsu Province, China

Received November 30, 2020;
Accepted February 10, 2021

ABSTRACT

Objective. We aimed to identify new candidate pathogenic genes for atypical Rolandic epilepsy.

Methods. We retrospectively evaluated the data from 24 Chinese patients with atypical Rolandic epilepsy who underwent whole-exome sequencing. Data were analysed regarding the frequency of affected genes, previously reported disease-related genes, and evaluation based on Kyoto Encyclopaedia of Genes and Genomes (KEGG).

Results. We identified a frameshift mutation in the reported gene *PRRT2*, which is classified as pathogenic according to American College of Medical Genetics and Genomics guidelines (ACMG). We also identified a novel missense mutation in the *PRRT2* gene in a family with three affected patients. Several other candidate genes were found in at least two patients, some of which were associated with other epilepsies (*ADGRV1, CACNA1A, CHD2, CLCN2, HECW2, KIF1A, NPRL3, RELN* and *TSC2*), while others were mainly associated with neuropsychiatric disease (*SHANK3* and *AUTS2*). The KEGG analysis of 81 candidate genes associated with atypical Rolandic epilepsy identified a significant association with the GABAergic synapse. Candidate genes involved in the GABAergic synapse pathway included *NSF*, *CACNA1A,* as well as others.

Significance. Our study indicates that *PRRT2* mutations may be associated with atypical Rolandic epilepsy. Moreover, we identified a number of unreported candidate genes, including *ADGRV1*, *CHD2*, *CACNA1A*, *NSF*, *NPRL3*, *KIF1A*, *GJB2* and *HECW2*, also associated with atypical Rolandic epilepsy.

Key words: atypical Rolandic epilepsy, candidate genes, whole-exome sequencing, *PRRT2*

Rolandic epilepsy (RE) is the most common genetic epilepsy syndrome in childhood, accounting for 15-24% of children with epilepsy, with an age at onset ranging from two to 13 years and a mean incidence of approximately seven years [1]. The prominent clinical features of "benign" Rolandic epilepsy, formerly named as "benign epilepsy with centrotemporal spikes (BECT)", include brief, focal, usually nocturnal seizures with sensorimotor symptoms, involving the face and laryngeal muscles, and secondary generalized tonic-clonic seizures. Centrotemporal spikes (CTS) are the hallmark of this syndrome. As the name indicates, BECT typically follows a benign, self-limiting course. However, 1-7% of the children exhibit atypical evolution, consisting of increased frequency of epileptic seizures, new types of seizures, cognitive and behavioural disturbances, and a spike-wave index (SWI) in slow-wave sleep in >50%; such cases are defined as atypical BECT [2]. Atypical BECT, Landau-Kleffner syndrome

• **Correspondence:**
Jihong Tang
No.92, Zhongnan Road,
Suzhou, Jiangsu Province,
215025, China
<tjhzsh_27@163.com>

doi:10.1684/epd.2021.1308

(LKS) and epileptic encephalopathy with continuous spike-and-waves during sleep syndrome (CSWS) are classified as clinical syndromes related to electrical status epilepticus in slow sleep (ESES) [2], and are also referred to as atypical Rolandic epilepsy (ARE) [3]. Unlike typical RE, ARE patients may experience intellectual impairment, behavioural disorders, and neurocognitive regression, even after adolescence [4]. RE and ARE are located at the opposite ends of a continuous spectrum of disorders, but are believed to share a common genetic basis [5]. Although the underlying genetic aetiologies remain largely unknown, there has been some recent progress in unravelling the genetic basis of the epilepsy spectrum [6, 7]. Some family studies have confirmed that CTS are associated with the *ELP4* gene [8]. *GRIN2A*, which is also an underlying disease-causing gene, but more relevant to speech disorders, has been detected in familial and sporadic cases, especially at the severe end of the RE/ARE spectrum [9] . Mutations in a series of voltage-gated potassium genes, including *KCNQ2*, *KCNQ3*, *KCNB1* and *KCNA2*, are risk factors for RE/ARE [10-12]. The voltage-gated sodium genes, *SCN2A* and *SCN9A,* have been reported to cause ESES-related syndrome and typical RE [13, 14]. A group of other candidate genes, including *PRRT2* and *SRPX2,* have been implicated in the pathogenicity of other brain disorders, such as paroxysmal kinesigenic dyskinesia (PKD), benign familial neonatal epilepsy (BFNE), and verbal dyspraxia [15, 16]. Furthermore, genomic alterations in *RBFOX*, *DEPDC5*, *SLC6A1* and *GABRG2* are also relevant risk factors [6, 17]. Finally, a recently published study has identified two novel disease-related genes: *GRIN2B* and *CAMK2A* [18]. Thus, previous genetic studies on ESES-related syndrome or RE/ARE indicate the existence of complex genetic mechanisms in this spectrum of diseases. However, these identified genes do not account for all known cases and it is not clear whether these genetic variants vary among different ethnicities in children. In this study, we aimed to:

- verify the known genes that are affected in Chinese patients;
- and explore potential candidate genes related to the RE/ARE spectrum through the use of whole-exome sequencing (WES) analysis in a cohort of patients with atypical Rolandic epilepsy.

Methods and materials

Study participants

Twenty-four patients were recruited from the outpatient and inpatient child neurology units of the Children's Hospital of Soochow University (n = 9) and Wuxi Children's Hospital (n = 15) from January 2016 to December 2018. The inclusion criterion for the present study was a diagnosis in accordance with the guidelines proposed by Scheltens-de Boer (2009) [2], as follows:

LKS:

- receptive/mixed aphasia, verbal agnosia;
- (infrequent) seizures;
- sleep stage with high SWI (unilateral/diffuse).

CSWS:

- global cognitive decline, motor disturbances;
- seizures;
- sleep stage with high SWI >85% or >50% (mostly diffuse).

Atypical BECT:

- cognitive and behavioural disturbances;
- mostly nocturnal (partial) seizures;
- sleep stage with high SWI >50% (focal/diffuse).

Exclusion criteria:

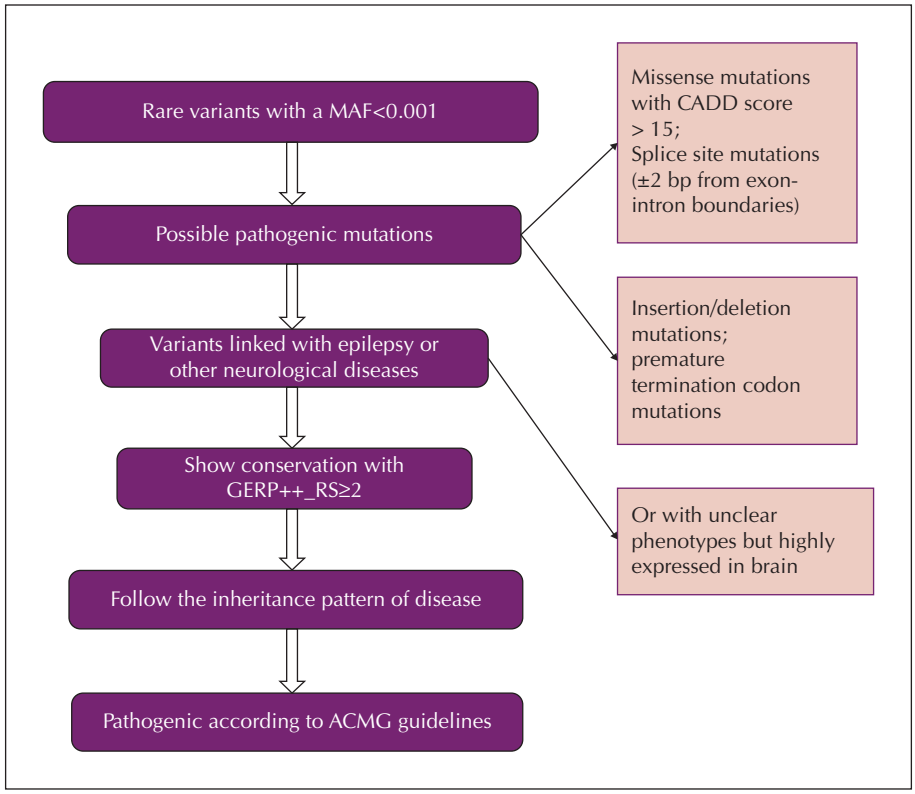
- ESES-related syndrome secondary to brain trauma, intracranial haemorrhage, hypoxic-ischemic encephalopathy and other factors;
- any other epilepsy syndromes accompanied by ESES, such as Lennox-Gastaut syndrome or West syndrome.

All patients underwent thorough neurological examinations and developmental evaluations. Patients' previous history and family history were recorded. Haematological examination and magnetic resonance imaging (MRI) of every patient showed no abnormalities. Electroencephalography (EEG) monitoring and genetic tests were performed for all patients. This study was approved by the Ethics Committee of the two hospitals. Written informed consent was obtained from the parents of all participants.

Genetic tests

Gene sample preparation and sequence analysis were completed by the Beijing Kangso Medical Inspection Center (China) and Kaiumph Medical Diagnostics Co, Ltd (China). Genomic DNA was extracted from venous blood samples of the patients and their parents using standard methods. WES analysis was performed in 24 patients using NextSeq 500 platform (Illumina, San Diego, CA, USA). Candidate genes/mutations were filtered according to the following criteria (*figure 1*):

- high-quality rare variants with a minor allele frequency (MAF) <0.001 in databases such as 1,000 genomes (http://browser.1000genomes.org), ExAC (http://exac.broadinstitute.org/), and ESP6500 (http://evs.gs.washington.edu/EVS);
- possible pathogenic missense mutations with CADD score >15, or splice site mutations



■ **Figure 1.** Flowchart of the analysis of candidate genes.

including 2 bp before and after the exon boundary position, or any insertion/deletion mutations, or premature termination codon mutations;
- variants linked with epilepsy or other neurological diseases, or with unclear phenotypes but highly expressed in the brain;
- conservation based on GERP++_RS≥2;
- an inheritance pattern of disease; and
- classified as pathogenic according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) [19].

If the variant was *de novo* or classified as pathogenic or likely pathogenic, but failed to meet Conditions 1, 2, or 4 of the criteria listed, it was also be retained as appropriate. Furthermore, mutations were verified by Sanger sequencing and segregation analysis was performed for families.

Results

Patient data

In the current study, we recruited 24 patients with ARE. Among them, one patient had LKS, two had CSWS, and the remaining patients had atypical BECT. All affected patients presented with epileptic seizures. Cognitive impairment was apparent and had developed prior to epilepsy onset in the case of CSWS. The patient with LKS experienced mixed aphasia. Twenty-one patients with atypical BECT experienced different degrees of cognitive, linguistic and behavioural disorders after the onset of seizures, with a severity that was lower than that in the patients with LKS or CSWS. All the participants were the children of non-consanguineous parents. Family history data up to four degrees were obtained from the proband and their parents. Overall, two (8.3%) of 24 probands had a positive family history of epilepsy. Age at onset ranged from two to nine years (median: six years). Five of the patients were female (20.8%), and 19 were male (79.2%) (*table 1*). SWI of slow-wave sleep >50% was observed on EEG in all patients.

Genetic data and KEGG analysis

Exome sequencing resulted in high-quality data with a mean coverage of 100×. According to the above-mentioned criteria, each patient had one to seven candidate genes. A total of 81 candidate genes and 101 mutations were detected (*supplementary table 1*). Among the 101 mutations, 82 (81.1%) were

▼ **Table 1.** Summary of clinical details of patients.

Patient number	Sex	Age at onset (years)	Family history	Clinical syndrome of ESES	SWI (%)	Developmental disorders	Seizure frequency	Treatment
1	M	8	None	Atypical BECT	75	Mild speech disorder	Infrequent	LEV
2	F	9	Paternal uncle had epilepsy	Atypical BECT	60	Mild inattention	Frequent	LEV, steroids
3	M	3	None	LKS	70	Mixed aphasia	Frequent	VPA, LEV, NZP, steroids
4	F	8	None	Atypical BECT	80	Mild inattention	Frequent	LEV, steroids
5	M	2	None	Atypical BECT	70	Hyperactivity	Frequent	VPA, steroids
6	M	7	None	Atypical BECT	80	Disturbances of memory	Frequent	OXC, LEV, NZP, steroids
7	M	3	None	CSWS	90	Severe speech disorder, hyperactivity, learning difficulty	Frequent	LEV, OXC, steroids
8	M	5	None	Atypical BECT	70	Cognitive impairment	Frequent	VPA, LEV, steroids
9	F	6	None	Atypical BECT	80	Impaired learning ability	Frequent	LEV, steroids
10	M	4	None	Atypical BECT	70	Impulsive, inattention, cognitive impairment	Frequent	VPA, LEV, steroids
11	M	6	None	Atypical BECT	55	Mild inattention	Frequent	VPA, CZP, steroids
12	M	7	None	Atypical BECT	80	Cognitive impairment, speech disorder	Frequent	VPA, LEV, CZP, steroids
13	M	6	None	Atypical BECT	70	Intellectual disability	Frequent	VPA, LEV, CZP, ssteroids
14	M	5	None	CSWS	80	Severe intellectual disability, gait and speech disturbance	Frequent	VPA, LEV, NZP, steroids
15	F	5	None	Atypical BECT	85	Mild speech disorder	Infrequent	VPA
16	M	7	None	Atypical BECT	55	Mild inattention	Infrequent	LEV
17	F	8	None	Atypical BECT	60	Mild cognitive impairment	Infrequent	LEV
18	M	7	None	Atypical BECT	50	Impaired ability to calculate	Infrequent	LEV

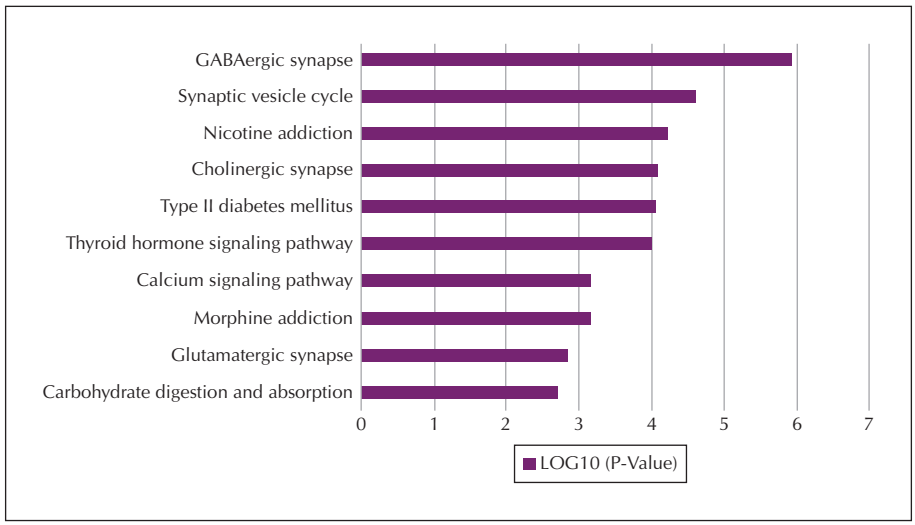
▼ **Table 1.** Summary of clinical details of patients (*continued*).

Patient number	Sex	Age at onset (years)	Family history	Clinical syndrome of ESES	SWI (%)	Developmental disorders	Seizure frequency	Treatment
19	M	6	None	Atypical BECT	65	Impaired ability to calculate	Infrequent	LEV
20	M	9	None	Atypical BECT	50	Speech disorder	Infrequent	VPA
21	M	6	None	Atypical BECT	60	Inattention	Infrequent	LEV
22	M	5	None	Atypical BECT	80	Impulsive	Infrequent	LEV
23	M	9	None	Atypical BECT	55	Inattention, impulsive	Infrequent	LEV
24	M	6	Grandfather and grandfather's sister had epilepsy	Atypical BECT	65	Learning difficulty, inattention	Frequent	VPA, CZP, steroids, LCM

Seizure frequency: frequent: >10 lifetime seizures; infrequent: <10 lifetime seizures; M: male; F: female; ESES: electrical status epilepticus in slow sleep; BECT: benign epilepsy of children with centrotemporal spikes; LKS: Landau–Kleffner syndrome; CSWS: continuous spike-and-waves during sleep syndrome; LEV: levetiracetam; VPA: valproate; NZP: nitrazepam; OXC: oxcarbazepine; CZP: clonazepam; LCM: lacosamid.

missense, two (2.0%) were nonsense, nine (8.9%) were frameshift mutations, four (4.0%) were deletion mutations, two (2.0%) were insertion mutations, and two (2.0%) were splice site mutations. Among these mutations, 85 (84%) were novel. Genes (*TSC2*, *TRIO*, *CACNA1H*, *NPRL3*) with *de novo* mutations were identified in four patients (16.6%) (*supplementary table 1*).

KEGG analysis of the 81 candidate genes revealed six genes (*CACNA1A, CACNA1B, CACNA1D, GABRD, NSF* and *SLC6A1*) involved in the GABAergic synapse pathway (*figure 2*).



■ **Figure 2.** KEGG analysis of 81 candidate genes in this study.

▼ **Table 2.** Detailed information of patients with *PRRT2* mutation.

Gene	No.	DNA mutation	Protein alteration	SIFT; Polyphen2; MutationTaster	CADD score	Novel	Max MAF	ACMG
RBFOX3	5	c.271G>A	p.Asp91Asn	-;Benign;Disease-causing	23.6	No	0.0002	US
PRRT2	5	c.649dupC	p.Arg217Profs*8	-	-	No	0.0344	Pathogenic
PRRT2	24	c.236C>T	p.Ser79Leu	Damaging, benign, polymorphism	23.11	Yes	0.0002	US
KCNB1	12	c.2506C>A	p.His836Asn	Probably damaging Damaging, disease-causing	25.9	Yes	0.00023	US
SLC6A1	20	c.589A>G	p.Met197Val	Tolerated, benign Disease-causing	17.13	Yes	0	US
KCNQ2	16	c.2602G>C	p.Ala868Pro	Tolerated, benign; Disease-causing	16.24	No	0.000005	US

Known RE/ARE-related genes

After comprehensive analysis, we identified six mutations within five genes (*table 2*) that have been previously reported to be associated with RE/ARE. Of the six mutations, *PRRT2*(c.649dupC) was classified as pathogenic according to ACMG standards. The other mutations were classified as "uncertain significance". A four-generation family with three affected members was enrolled in this study (*figure 3A*). Exome sequencing revealed a novel missense mutation (NM_145239 c.236C>T) in heterozygous form within the *PRRT2* gene in Patient 24 (*figure 3B*) and his grandfather, as well as his unaffected father.

Prevalence of affected genes

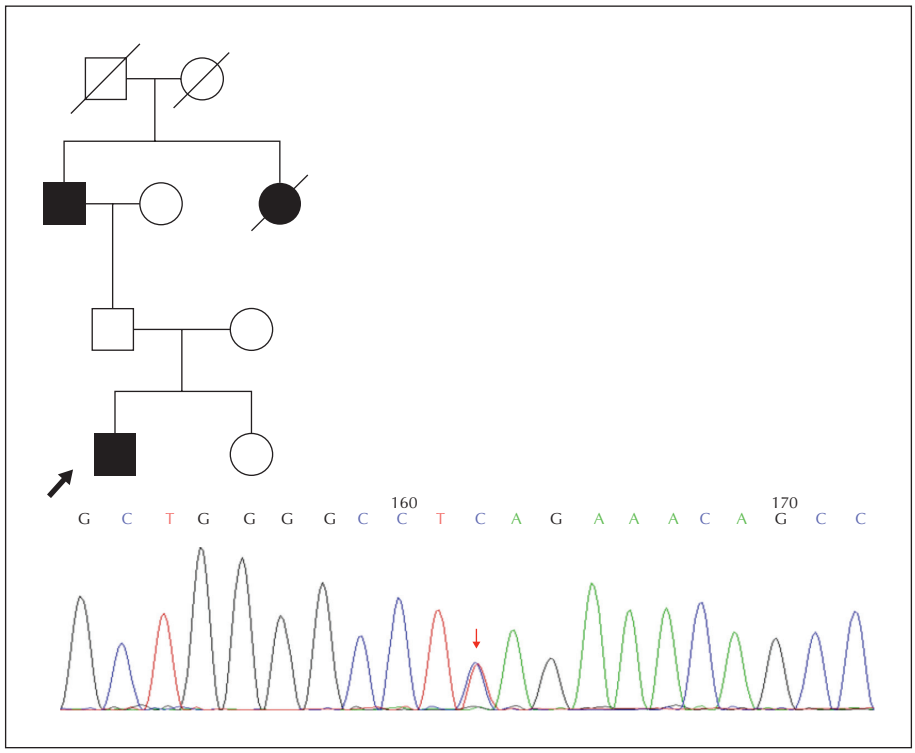
Genes that were affected in more than one patient and identical mutations, that satisfied the criteria described above, were analysed. The most commonly affected gene was *CACNA1A*, which was present in four patients. Other genes mutated in more than one patient included three genes (*TSC2, SHANK3* and *HECW2*) in three patients and 11 genes (*AUTS2, ADGRV1, BSN, CDH2, CLCN2, GJB2, INTS1, KIF1A, PRRT2, RELN, TAS2R19*) in two patients (*figure 4*). Some mutations in *PRRT2, HECW2* and *GJB2* were classified as pathogenic or likely pathogenic according to ACMG standards.

In addition, identical mutations were detected in three genes: p. Arg2294Pro (*CACNA1A*), p. Glu917_Glu917del (*KIF1A*) and p. Thr181Thrfs*24 (*TAS2R19*). All these mutations were classified as "uncertain significance".

Discussion

In the present study, we performed WES in 24 unrelated Chinese patients with ARE in order to identify potentially causative genes and support previous data. Overall, in this study, only five genes detected in five patients were consistent with previously reported causal genes, indicating the heterogeneity and complexity of the disease. *PRRT2* is one of these few genes. Dimassi *et al*. [15] reported genomic alterations in *PRRT2*, detected in two patients with ARE. In addition, Torisu [20] reported a case of a Japanese girl with a hot spot *PRRT2* mutation (c.649_650insC, also recorded as c.649dupC) who developed focal seizures with spikes resembling Rolandic spikes. In our study, the same heterozygous mutation, c.649dupC, and a novel mutation, c.236C > T, in *PRRT2* were detected in two patients with ARE, respectively. One of these was rated as pathogenic according to ACMG guidelines, and the other was carried in two affected members of the family, suggesting that *PRRT2* gene mutation may be related to the pathogenesis of RE/ARE. However, individuals with the same genotype ultimately showed different degrees of disease or clinical symptoms. A comparison of the electroclinical features of cases with *PRRT2* mutation is presented in *table 3*. In this study, the *PRRT2* gene mutation of Patient 5 was derived from his healthy mother, and that of Patient 24 was derived from his healthy father, which may be due to the incomplete penetrance of the gene. In addition to *PRRT2*, we also identified other gene variants inherited from unaffected parents, indicating that incomplete penetrance may be common in RE/ARE as previously reported in the literature [21].

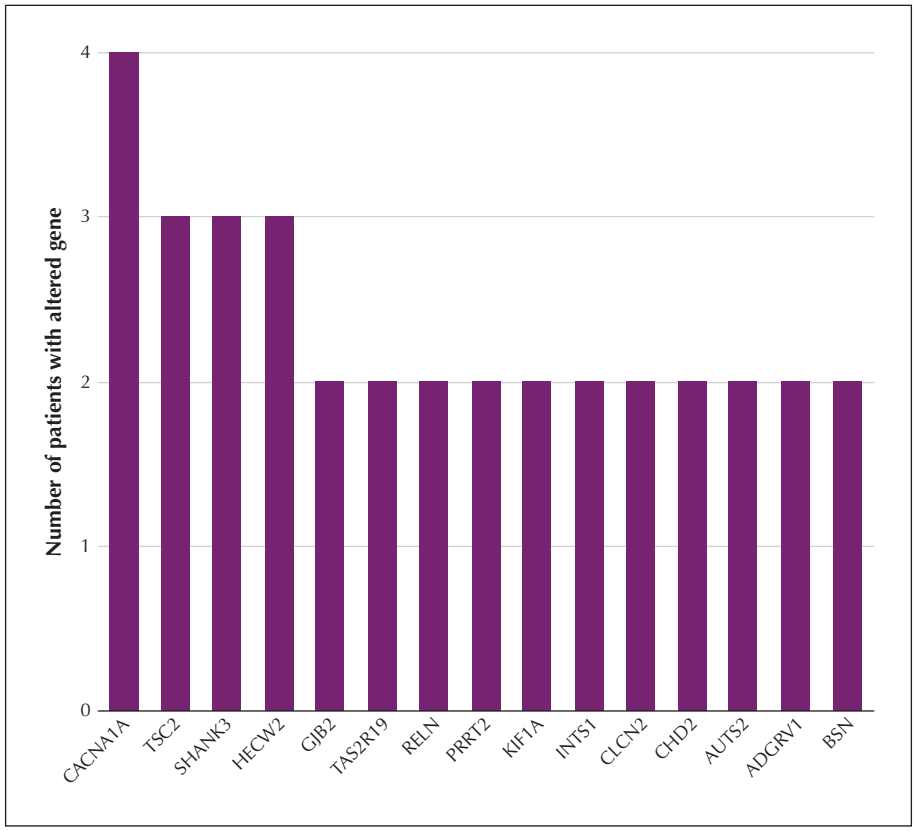
The number of genes affected in at least two patients was relatively small. The most frequently affected



■ **Figure 3.** (A) The pedigree of Patient 24's family. Filled-in symbols indicate individuals with epilepsy, empty symbols indicate unaffected individuals, and symbols with a slash indicate deceased individuals. Arrow refers to the proband (Patient 24). (B) Identification of a heterozygous mutation, c.236C>T, of the *PRRT2* gene in the proband.

gene was *CACNA1A*, in which heterozygous mutations were identified in four patients. Variants in *CACNA1A* and another gene, *CLCN2* (found in two patients), which encode for subunits of voltage-gated ion channels, have been widely reported in a variety of epilepsy syndromes. *TSC2* was identified in three patients, and the *de novo* frameshift mutation in Patient 23 highlights this gene as a candidate gene of interest. *TSC2*, the previously reported causal gene *DEPDC5*, as well as the *de novo* mutated *NPRL3* gene in this study are all mTOR-signalling regulatory genes that are highly associated with malformations of cortical development [22, 23]. Pacheva *et al*. [24] reported the case of a 13-year-old girl with tuberous sclerosis complex, who presented with severe epilepsy, autism and ESES on EEG. Coincidentally, in the recently published study of Rudolf *et al*. [18], a *de novo* variant of *TSC2* was also found in a patient with ARE, moreover, the authors also identified *ADGRV1* variants, which were found in two patients in this study. *RELN* was identified as a candidate gene for LKS in the study conducted by Conroy *et al*. [25], as well as *BSN*, of which variants were found in two patients in our study. *CHD2* is another epilepsy-related gene that should be considered as a candidate gene for ARE. In addition to various forms of epilepsy, *CHD2* is also associated with cognitive and behavioural abnormalities [26]. The genes, *SHANK3* and *AUTS2*, are typically linked to neuropsychiatric disorders. However, a mutation in *SHANK3* was previously identified in a patient with CSWS, and it was suggested that the two spectrum diseases have some genetic basis in common [27]. Other gene variants, such as those of *HECW2* and *KIF1A*, have been reported to be associated with epilepsy and intellectual disability [28, 29].

The KEGG analysis in our study revealed that the mutated genes are largely specific to the GABAergic synapse pathway. GABA is the main inhibitory neurotransmitter in the brain and the target of many antiepileptic drugs. Diazepam, the first-line drug of choice for CSWS in North America [30], also acts on the GABAergic pathway. Previous studies have shown that corticosteroids act on GABA receptors and increase GABA levels in cerebrospinal fluid, producing an anticonvulsant effect [31], which may be the mechanism in which corticosteroids function as treatment for ARE. This theory may shed light on the pathogenesis of ESES and help guide treatment.



■ **Figure 4.** Prevalence of genes mutated in the cohort of 24 RE/ARE patients.

▼ **Table 3.** Specific details of previously identified genes detected in the study.

	Patient 1	Patient 2	Patient 3
Origin	Present study	Present study	Torisu et al [20]
Mutation	*PRRT2* c.649dupC (p. Arg217Profs*8)	*PRRT2* c.236C>T (p. Ser79Leu)	*PRRT2* c.649_650insC (p. Arg217Profs*8)
Family history	Same mutation in mother with no symptoms	Father was not affected, but grandfather had epilepsy	Father had PKD and youngest sister had benign infantile seizures
Diagnosis	Atypical BECT	Atypical BECT	Infantile focal epilepsy
Phenotype	Generalized tonic-clonic seizures	Clusters of focal seizures, generalized tonic-clonic seizures, intractable epilepsy	Focal epilepsy, generalized tonic seizure
Onset Age	2 years	6 years	14 months
EEG	Discharges in Rolandic region, ESES (SWI 70%)	Discharges in rolandic region, ESES (SWI 65%)	Multifocal spikes in bilateral centrotemporal areas, moving transiently to the occipital area, resembling Rolandic spikes
MRI	Normal	Normal	Normal
Comorbidity	Hyperactivity	Learning difficulty, inattention	None
Treatment	VPA, steroids	VPA, CZP, steroids, LCM	CBZ
Response to Treatment	Good	Good	Good

ESES: electrical status epilepticus in slow sleep; BECT: benign epilepsy of children with centrotemporal spikes; VPA: valproate; CZP: clonazepam; LCM: lacosamide; CBZ: carbamazepine; PKD: paroxysmal kinesigenic dyskinesia.

This study has some limitations. Firstly, we did not include mutations in untranslated regions (UTRs) which may also be disease-causing. Furthermore, the sample size of this study was small, and copy number variation was not performed, thus only few genes with strong pathogenicity were found. Despite its limitations, the present study provides important insight into the candidate genes responsible for ARE.

In conclusion, in addition to some previously reported RE/ARE-related gene mutations, a number of novel candidate genes, such as *ADGRV1, PRODH, CHD2, CACNA1A, NSF, NPRL3, KIF1A, HECW2,* amongst others, were detected in this study. *PRRT2* mutations have been shown to be associated with atypical Rolandic epilepsy. The common *GRIN2A* mutation in this disease spectrum was absent in our cohort of Chinese patients. Compared with previous studies, the number of genes affected in more than one patient was small, which demonstrates the genetic heterogeneity of the disease. KEGG analysis of candidate genes suggests the involvement of the GABAergic pathway in atypical Rolandic epilepsy. It is hoped that newly discovered candidate genes will improve our knowledge, and lead to more targeted therapeutic approaches for some ARE patients. ■

Supplementary material.
Supplementary table and summary slides accompanying the manuscript are available at www.epilepticdisorders.com.

Acknowledgements and disclosures.
This work was supported by the Science and Technology Program of Suzhou (grant number SS201866), Science and Technology Project of the Health and Health Committee of Jiangsu Province(grant number H2018010), Wuxi Maternal and Child Health Research Project (grant number FYKY201904), and Wuxi Taihu Lake Talent Plan Top Talents Project (grant number HB2020086). None of the authors have any conflicts of interest to declare.

Ethical approval permission number.
WXCH2015-12-004

References

1. Vannest J, Tenney JR, Gelineau-Morel R, Maloney T, Glauser TA. Cognitive and behavioral outcomes in benign childhood epilepsy with centrotemporal spikes. *Epilepsy Behav* 2015; 45: 85-91.

2. Scheltens-de Boer M. Guidelines for EEG in encephalopathy related to ESES/CSWS in children. *Epilepsia* 2009; 50(Suppl 7): 13-7.

3. Fejerman N. Atypical rolandic epilepsy. *Epilepsia* 2009; 50(Suppl 7): 9-12.

4. Pesantez-Rios G, Martinez-Bermejo A, Pesantez-Cuesta G. Neuropsychological alterations are frequent in rolandic epilepsy and its atypical developments. *Rev Neurol* 2016; 63(3): 125-9.

5. Rudolf G, Valenti MP, Hirsch E, Szepetowski P. From rolandic epilepsy to continuous spike-and-waves during sleep and Landau-Kleffner syndromes: insights into possible genetic factors. *Epilepsia* 2009; 50 (Suppl 7): 25-8.

6. Xiong W, Zhou D. Progress in unraveling the genetic etiology of rolandic epilepsy. *Seizure* 2017; 47: 99-104.

7. Kessi M, Peng J, Yang L, Xiong J, Duan H, Pang N, *et al*. Genetic etiologies of the electrical status epilepticus during slow wave sleep: systematic review. *BMC Genet* 2018; 19(1): 40.

8. Strug LJ, Clarke T, Chiang T, Chien M, Baskurt Z, Li W, *et al*. Centrotemporal sharp wave EEG trait in rolandic epilepsy maps to Elongator Protein Complex 4 (ELP4). *Eur J Hum Genet* 2009; 17(9): 1171-81.

9. Lesca G, Rudolf G, Bruneau N, Lozovaya N, Labalme A, Boutry-Kryza N, *et al*. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. *Nat Genet* 2013; 45(9): 1061-6.

10. Neubauer BA, Waldegger S, Heinzinger J, Hahn A, Kurlemann G, Fiedler B, *et al*. KCNQ2 and KCNQ3 mutations contribute to different idiopathic epilepsy syndromes. *Neurology* 2008; 71(3): 177-83.

11. de Kovel CGF, Syrbe S, Brilstra EH, Verbeek N, Kerr B, Dubbs H, *et al*. Neurodevelopmental disorders caused by de novo variants in KCNB1 genotypes and phenotypes. *JAMA Neurol* 2017; 74(10): 1228-36.

12. Masnada S, Hedrich UBS, Gardella E, Schubert J, Kaiwar C, Klee EW, *et al*. Clinical spectrum and genotype-phenotype associations of KCNA2-related encephalopathies. *Brain* 2017; 140(9): 2337-54.

13. Wolff M, Johannesen KM, Hedrich UBS, Masnada S, Rubboli G, Gardella E, *et al*. Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain* 2017; 140(5): 1316-36.

14. Liu Z, Ye X, Qiao P, Luo W, Wu Y, He Y, *et al*. G327E mutation in SCN9A gene causes idiopathic focal epilepsy with Rolandic spikes: a case report of twin sisters. *Neurological Sci* 2019; 40(7): 1457-60.

15. Dimassi S, Labalme A, Lesca G, Rudolf G, Bruneau N, Hirsch E, *et al*. A subset of genomic alterations detected in rolandic epilepsies contains candidate or known epilepsy genes including GRIN2A and PRRT2. *Epilepsia* 2014; 55(2): 370-8.

16. Roll P, Rudolf G, Pereira S, Royer B, Scheffer IE, Massacrier A, *et al*. SRPX2 mutations in disorders of language cortex and cognition. *Human Mol Genet* 2006; 15(7): 1195-207.

17. Johannesen KM, Gardella E, Linnankivi T, Courage C, de Saint Martin A, Lehesjoki AE, *et al*. Defining the phenotypic spectrum of SLC6A1 mutations. *Epilepsia* 2018; 59(2): 389-402.

18. Rudolf G, de Bellescize J, de Saint Martin A, Arzimanoglou A, Valenti Hirsch MP, Labalme A, *et al*. Exome sequencing in 57 patients with self-limited focal epilepsies of childhood

with typical or atypical presentations suggests novel candidate genes. *Eur J Paediatr Neurol* 2020; 27: 104-10.

19. 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(5): 405-24.

20. Torisu H, Watanabe K, Shimojima K, Sugawara M, Sanefuji M, Ishizaki Y, *et al*. Girl with a PRRT2 mutation and infantile focal epilepsy with bilateral spikes. *Brain Dev* 2014; 36(4): 342-5.

21. Addis L, Sproviero W, Thomas SV, Caraballo RH, Newhouse SJ, Gomez K, *et al*. Identification of new risk factors for rolandic epilepsy: CNV at Xp22.31 and alterations at cholinergic synapses. *J Med Genet* 2018; 55(9): 607-16.

22. Crino PB. mTOR signaling in epilepsy: insights from malformations of cortical development. *Cold Spring Harb Perspect Med* 2015; 5(4): a022442.

23. 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.

24. Pacheva I, Panov G, Gillberg C, Neville B. A girl with tuberous sclerosis complex presenting with severe epilepsy and electrical status epilepticus during sleep, and with high-functioning autism and mutism. *Cogn Behav Neurol* 2014; 27(2): 88-95.

25. Conroy J, McGettigan PA, McCreary D, Shah N, Collins K, Parry-Fielder B, *et al*. Towards the identification of a genetic basis for Landau-Kleffner syndrome. *Epilepsia* 2014; 55(6): 858-65.

26. Bernardo P, Galletta D, Iasevoli F, D'Ambrosio L, Troisi S, Gennaro E, *et al*. CHD2 mutations: only epilepsy? Description of cognitive and behavioral profile in a case with a new mutation. *Seizure* 2017; 51: 186-9.

27. Lesca G, Rudolf G, Labalme A, Hirsch E, Arzimanoglou A, Genton P, *et al*. Epileptic encephalopathies of the Landau-Kleffner and continuous spike and waves during slow-wave sleep types: genomic dissection makes the link with autism. *Epilepsia* 2012; 53(9): 1526-38.

28. Ullman NL, Smith-Hicks CL, Desai S, Stafstrom CE. De novo HECW2 mutation associated with epilepsy, developmental decline, and intellectual disability: case report and review of literature. *Pediatr Neurol* 2018; 85: 76-8.

29. Kurihara M, Ishiura H, Bannai T, Mitsui J, Yoshimura J, Morishita S, *et al*. A novel de novo KIF1A mutation in a patient with autism, hyperactivity, epilepsy, sensory disturbance, and spastic paraplegia. *Intern Med* 2019; 59(6): 839-42.

30. Sanchez Fernandez I, Chapman K, Peters JM, Klehm J, Jackson MC, Berg AT, *et al*. Treatment for continuous spikes and waves during sleep (CSWS): survey on treatment choices in North America. *Epilepsia* 2014; 55(7): 1099-108.

31. Rogawski MA, Reddy DS. Neurosteroids and infantile spasms: the deoxycorticosterone hypothesis. *Int Rev Neurobiol* 2002; 49: 199-219.

TEST YOURSELF

(1) Which syndrome is not considered as atypical Rolandic epilepsy?

A. Atypical BECT
B. Landau–Kleffner syndrome (LKS)
C. Epileptic encephalopathy with continuous spike-and-waves during sleep syndrome (CSWS)
D. Lennox-Gastaut syndrome (LGS)

(2) Variants in which of the following potassium genes are considered to be related to the pathogenesis of atypical Rolandic epilepsy or Rolandic epilepsy?

A. KCNA2
B. KCNQ2
C. KCNQ3
D. KCNB1

(3) What clinical phenotypes can *PRRT2* mutations cause?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".