

Clinical and genetic analysis of 18 patients with *KCNQ2* mutations from South China

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ABSTRACT

Background. We aimed to delineate the genotype and phenotype of patients with *KCNQ2* mutations from South China.

Methods. Clinical manifestations and characteristics of *KCNQ2* mutations of patients from South China were analyzed. Previous patients with mutations detected in this study were reviewed.

Results. Eighteen epilepsy patients with *KCNQ2* mutations, including seven self-limited neonatal epilepsy (SeLNE), two self-limited infantile epilepsy (SeLIE) and nine developmental and epileptic encephalopathy (DEE) were enrolled. The age of onset ($p=0.006$), mutation types ($p=0.029$), hypertonia ($p=0.000$), and seizure offset ($p=0.029$) were different in self-limited epilepsy (SeLE) and DEE. *De novo* mutations were mainly detected in DEE patients ($p=0.026$). The mutation position, EEG or the age of onset were not predictive for the seizure or ID/DD outcome in DEE, while the development of patients free of seizures was better than that of patients with seizures ($p=0.008$). Sodium channel blockers were the most effective anti-seizure medication, while the age of starting sodium channel blockers did not affect the seizure or development offset. We first discovered the seizure recurrence ratio in SeLNE/SeLIE was 23.1% in South China. Four novel mutations (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G, 20q13.33del) were discovered. Each of eight mutations (c.1918delC, c.1678C>T, c.683A>G, c.833T>C, c.868G>A, c.638G>A, c.997C>T, c.830C>T) only resulted in SeLE or DEE, while heterogeneity was also found. Six patients in this study have enriched the known phenotype caused by the mutations (c.365C>T, c.1A>G, c.683A>G, c.833T>C, c.830C>T, c.1678C>T).

Conclusion. This research has expanded known phenotype and genotype of *KCNQ2*-related epilepsy, and the different clinical features of SeLE and DEE from South China.

Key words: *KCNQ2*, self-limited epilepsy, developmental and epileptic encephalopathy.

KCNQ2 (OMIM *602235), encoding a voltage-gated potassium channel, was firstly reported to be a disease-causing gene for epilepsy in 1998.¹ To date, heterozygous mutations in *KCNQ2* are considered responsible for a spectrum of disease, ranging from self-limited neonatal epilepsy (SeLNE), self-limited infantile epilepsy (SeLIE) to developmental and epileptic encephalopathy (DEE, OMIM #613720).^{2,3} Various types of mutations have

been reported.^{4,5} In the Human Gene Mutation Database, more than 400 variations in *KCNQ2* are recorded. Early onset seizures without intellectual disability/development delay (ID/DD) are common manifestations in SeLNE and SeLIE, they might spontaneously disappear or are pharmacoresponsive.⁶ SeLNE/SeLIE patients also have a probability to progress into epilepsy again after late childhood.⁷ DEE, in contrast, is characterized by seizures onset in the first week of life and unremitting moderate or severe ID/DD. Seizures are often resistant to regular anti-seizure medications (ASMs), and may cease between nine months and four years of age.³ Loss of function, dominant negative effects and gain of function have been

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confirmed as the underlying mechanisms.^{4,8,9} Sodium channel blockers have been found to be the most effective ASM.¹⁰

Recent clinical studies have described the clinical features of *KCNQ2* related diseases, including manifestations, treatment methods and prognosis.^{8,11} However, there are few studies reporting patients with *KCNQ2* mutations from South China, especially from Guangdong Province, and the electroclinical features have not yet been clarified. In this study, we aimed to explore the genetic and clinical features of eighteen epilepsy patients with *KCNQ2* mutation from South China and analyze and compare the clinical manifestations of children with the same mutation in this and previous studies.

Methods

Patients

Eighteen patients (Pt1-Pt18) with epilepsy onset in infancy and *KCNQ2* variations from 18 unrelated families were enrolled at the Department of Pediatric Neurology, Guangzhou Women and Children's Medical Center between June 2018 and August 2021. Our study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (No. 2019-40101). We have obtained informed consent from the patients' parents for the study.

Clinical analysis

Clinical data were collected, including age of onset, gender, seizure type, treatment method, developmental milestones, EEG and MRI. Before the era of genetic testing, treatment methods of 18 patients in this study were based on age, seizure type, and EEG characteristics. Since some patients had started their evaluation and treatment in other hospitals, treatment regimens were heterogeneous. Vitamin B6 was applied in some patients irregularly, and therefore was not analyzed for effectiveness in this study. Response to ASMs was considered effective if

seizure frequencies decreased by more than 50% from the baseline after treatment. For the sake of simplicity in this study, developmental assessment was based on developmental milestones and ID/DD was classified as mild, moderate or severe based on the following: mild ID/DD was considered when patients acquired the developmental milestones but at a slightly worse level. Moderate ID/DD was defined as patients who were obviously delayed in milestones, but who showed progress. Patients were classified as severe or profound ID/DD if their development was nearly arrested. The descriptions of seizure and epilepsy were based on the guidelines proposed by the International League Against Epilepsy in 2017 and 2022.^{12,13}

Genetic analysis

Next-generation sequencing including epilepsy gene panel sequencing, whole exome sequencing and whole genome sequencing was performed for 18 patients in genetic testing agencies. The epilepsy gene panel was designed to capture the coding exons of more than 536 genes associated with epilepsy including the *KCNQ2*. Next-generation sequencing was performed on Illumina, NovaSeq 6000 or MGI 2000 system platforms. Sequences of genes were referred to GRCh37/hg19. The average sequencing depth of epilepsy gene panels and whole exome sequencing was more than 122X. The average sequencing depth of whole genome sequencing was 28X. Variations of eleven patients (Pt2-Pt4, Pt6, Pt8, Pt10, Pt12-Pt15, Pt17) were validated with Sanger sequencing. All *KCNQ2* variants were harmonized with the ENST00000359125.7 (RefSeq NM_172107.4) of the GRCh37/hg19 human reference genome build. Variants were analyzed according to the recommendations of the American College of Medical Genetics and Genomics (ACMG).^{14,15}

Review of previous patients

Clinvar database, Human Gene Mutation Database and the literature (Supplementary table) were reviewed to analyze the clinical course, seizure types, and response to the

treatment of patients with the same mutation detected in this study.^{8,11,16-40}

Statistical analysis

Data were evaluated by Fisher exact probability test, Mann Whitney U test, and Kruskal-Wallis H test for comparisons between groups using Statistical Package for the Social Sciences version 20 software. Probability values less than 5% were considered to be significant.

Results

Clinical features

Eighteen children (Pt1-Pt18) from South China, composed of eight female and ten males, were clinically evaluated in this study (Table I). Seven patients (Pt1-Pt7) were diagnosed with SeLNE, two (Pt8-Pt9) were SeLIE, and nine (Pt10-Pt18) were DEE. Fifteen patients came from the Guangdong Province, two from Guangxi (Pt2 and Pt8), and one from the Hunan (Pt3) Province. None of their parents were consanguineous. Pt17 was born prematurely at 36 weeks without asphyxia. Gestational diabetes and maternal thyroid dysfunction were diagnosed in Pt4 and Pt13, respectively. No specific abnormalities were found in blood routine test or metabolic disease screening in eighteen patients. In the brain MRI, smaller right hippocampus was found in Pt2, and diffuse atrophy was detected in Pt14 and Pt17. A positive family history of seizure was found in three patients (Pt1, Pt4 and Pt6). Seizures in three family members of two SeLNE patients (Pt4, Pt6) disappeared spontaneously without treatment in infancy. Seizures recurred to Pt1's father during adulthood.

Seizure was the initial symptom and was observed in all of them (Table I). Patients of SeLE developed seizures from 3 days to 3 months after birth, with a median of 5 days, while, in DEE patients, seizures occurred at the age ranging from several hours to six months of age, with a median of 2 days. The age of onset of DEE patients was earlier than that of self-

limited epilepsy (SeLE) patients ($p=0.006$) (Table II). Focal onset seizure was the most common seizure type (88.9%, 16/18).

EEG often presented focal or multi-focal discharge in both SeLE and DEE patients (94.4%, 17/18), while suppression burst patterns and hypsarrhythmia were only detected in DEE patients ($p=0.000$) (see Table I and Table II). Initial epilepsy syndrome was Ohtahara syndrome in eight patients and West syndrome in one patient. Four patients of Ohtahara syndrome evolved to West syndrome. In eight patients with ictal amplitude-integrated electroencephalography (aEEG) in neonate, a sudden rise of the upper and lower margin followed by a marked depression in amplitude were found in eight and three, respectively.

ASMs (18/18), prednisone (3/18), and ketogenic diet (2/18) were used in this study. Generally, sodium channel blockers (SCBs), including oxcarbazepine (100%, 15/15), lamotrigine (100%, 3/3), and topiramate (66.7%, 2/3) were used and effective in 100% of patients ($n=15$) and its usage rate was higher in DEE patients than that in SeLE ($p=0.029$). In seven SeLNE patients, phenobarbital (5/7), valproic acid (5/7), topiramate (1/7), levetiracetam (1/7), nitrazepam (1/7) and oxcarbazepine (5/7) were used, in which, phenobarbital (100%, 5/5) and SCBs (100%, 6/6) were the most effective, followed by valproic acid (50%, 2/4). Lacosamide was tried in Pt3, but was too early to evaluate the effectiveness as it was only recently started during the preparation of this manuscript. They were pharmaco-responsive and became seizure-free in infancy, with a median age of 3+ months old. Oxcarbazepine, levetiracetam and valproic acid were effective in two patients with SeLIE, and both of them were free of seizures in their infancy. Nine DEE patients were responsive to SCBs, especially oxcarbazepine (100%, 9/9). In addition, nitrazepam (3/3), valproic acid (6/7), and phenobarbital (3/6) were effective in most of them. A combination of prednisone and other ASMs was found to be effective in three West syndrome patients. However, levetiracetam (0/6), vigabatrin (0/1) and ketogenic diet (0/2)

Table I. Genetic and clinical features of 18 patients with mutations from South China

Pt	Gender	Mutation/origin	Age of onset	Seizure	aEEG	EEG	Treatment		Diagnosis	Age at last visit	Prognosis
							Ineffective	Effective			
1	M	c.394G>A(p.Val132Met), Father	3d	Focal (FBTCS, TS)	/	IP: MFD→Normal	VPA	OXC	SeLNE	2y1m	SF since 3m with OXC (reduction)
2	M	c.1741C>T(p.Arg581*), <i>De novo</i>	6d	Focal (FBTCS)	/	IP: Asymmetric EW in TL→Normal	/	PB, VPA	SeLNE	2y1m	SF since 4m with VPA (reduction)
3	M	c.790T>C(p.Tyr264His), <i>De novo</i>	5d	Focal (TCS, FBTCS)	/	IP: MFD	/	TPM, OXC	SeLNE, EP	2y	SF since 5m with OXC
			3y11m	Focal (FBTCS)	/	IP: MFD	LCM(?)			4y	Occasional Sz with OXC+LCM
4	M	c.1918delC(p.Leu640Trpfs*1), Mother	7d	Focal (TCS, FBTCS)	RM	IP: MFD→Normal	/	PB, OXC	SeLNE	3y1m	SF since 5m
5	F	c.355_363delGAGAGAGCC (p.Glu119_Ser121del), <i>De novo</i>	3d	Focal (FBTCS)	RM, DA	IP: Sparse EW	/	PB, OXC	SeLNE	6m	SF since 10+d with OXC
6	F	c.296+2T>G, Mother	3d	Focal (FBTCS)	/	IP: Sparse EW →Normal/		PB, VPA	SeLNE	1y9m	SF since 3m with VPA
7	M	20q13.33del(chr20:61974641-62324656)*1,350kb, NA	3d	Focal (TS, FBTCS)	RM	IP: MFD	VPA, LEV, PB, OXC		SeLNE	7m	SF since 3m with OXC
8	M	c.1A>G(p.Met1Val), NA	3+m	Unknown (TCS)	/	NA	NA	OXC	SeLIE, EP	NA	SF
			8y2m	Unknown (TCS)	/	IP: MFD	NZP	LEV		9y	Occasional Sz with NZP+LEV
9	M	c.998G>A(p.Arg333Gln), Father	2+m	Unknown (TCS)	/	IP: MFD→Normal	/	LEV, VPA	SeLIE	2y10m	SF since 5m with VPA
10	F	c.1678C>T(p.Arg560Trp), <i>De novo</i>	2d	Focal and General (FBTCS, TS, Spm) (GESELL, 2m:42)	/	IP: MFD→SB→HS, IP: MFD→IP:EW	LEV	PB, OXC, PDN	DEE, OS, WS	1y9m	SF and moderate ID/DD since 5m with OXC+PB
11	F	c.683A>G(p.His228Arg), <i>De novo</i>	2d	Focal and General (FBTCS, Spm)	RM	SB→HS, IP: MFD→IP: MFD	PB, LEV, VGB	OXC, NZP, PDN, VPA, WS	DEE, OS, WS	1y7m	Infrequent Sz and profound ID/DD with OXC+VPA+NZP+LTG
12	F	c.833T>C(p.Ile278Thr), <i>De novo</i>	2d	Sz: Focal and General (TS, FBTCS, Spm, CS) (GESELL 6m:10)	RM	SB, IP: MFD→HS, IP: MFD	/	PB, TPM, OXC, LTG, VPA, PDN	DEE, OS, WS	2y1m	Sz sometimes and profound ID/DD with VPA+OXC+LTG

Pt, patient; h, hours old; d, days old; w, weeks old; m, months old; y, years old; F, female; M, male; CS, clonic seizures; DA, a marked depression in amplitude; EP, epilepsy; EW, epileptic waves; FBTCS, focal to bilateral tonic-clonic seizure; HS, hypersarrhythmia; IP, interictal period; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; MFD, multifocal discharges; NA, not available; /, No; NZP, nitrazepam; OXC, oxcarbazepine; PB, phenobarbital; PDN, prednisone; RM, rise of the upper and lower margin; SB, suppression burst; SF, seizures free; Spm, spasm; Sz, seizures; TCS, tonic-clonic seizures; TL, temporal lobe; TPM, topiramate; TS, tonic seizures; VGB, vigabatrin; VPA, valproic acid; WES, whole exon sequencing; WGS, whole genome sequencing.

Table I. Continued.

Pt	Gender	Mutation/origin	Age of onset	Seizure	aEEG	Treatment		Diagnosis	Prognosis	
						Ineffective	Effective		Age at last visit	Condition
13	M	c.365C>T(p.Ser122Leu), <i>De novo</i>	8h	Focal and General (TS, FBTCs)	RM	PB, VPA	OXC	DEE, OS	1y2m	SF and mild ID/DD since 2m with OXC
14	F	c.1678C>T(p.Arg560Trp), <i>De novo</i>	2d	Focal and General (TS, Spm)	/	LEV, KD	VPA, LTG, OXC, NZP	DEE, OS	1y1m	Frequent Sz and profound ID/DD with VPA+LTG+OXC+NZP
15	F	c.868G>A(p.Gly290Ser), NA	1d	Focal and General (TS, Spm)	/	TPM, LEV	NZP, VPA, OXC	DEE, OS	1y6m	SF and moderate ID/DD since 3m with OXC
16	M	c.638G>A(p.Arg213Gln), <i>De novo</i>	2d	Focal and General (TS, TCS, Spm)	RM, DA	PB	VPA, OXC	DEE, OS, WS	3m	Frequent Sz and profound ID/DD with VPA+OXC
17	M	c.997C>T(p.Arg333Trp), <i>De novo</i>	6m	Focal and General (Spm, TS)	/	LEV, KD	VPA, OXC	DEE, WS	1y5m	Infrequent Sz and profound ID/DD with VPA+OXC
18	F	c.830C>T(p.Thr277Ile), <i>De novo</i>	2d	Focal and General (TCS, TS, Spm)	RM, DA	LEV	PB, OXC	DEE, OS	6m	SF and moderate ID/DD since 3+m with OXC+PB

Pt, patient; h, hours old; d, days old; w, weeks old; m, months old; y, years old; F, female; M, male; CS, clonic seizures; DA, a marked depression in amplitude; EP, epilepsy; EW, epileptic waves; FBTCs, focal to bilateral tonic-clonic seizure; HS, hypsarrhythmia; IP, interictal period; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; MFD, multifocal discharges; NA, not available; /, No; NZP, nitrazepam; OXC, oxcarbazepine; PB, phenobarbital; PDN, prednisone; RM, rise of the upper and lower margin; SB, suppression burst; SF, seizures free; Spm, spasms; Sz, seizures; TCS, tonic-clonic seizures; TL, temporal lobe; TPM, topiramate; TS, tonic seizures; VGB, vigabatrin; VPA, valproic acid; WES, whole exon sequencing; WGS, whole genome sequencing.

Table II. Genotype and phenotype correlation.

Items	Phenotype		T	p	Seizure offset in DEE			T	p	Neurodevelop. In DEE			T	p
	SeLE	DEE			Yes	No	2d			Mild	Moderate	Profound		
Age of onset	5d	2	17 ¹	0.006*	1.5d	8h	2d	9	0.081*	8h	2d(1d-2d)	2d(2d-6m)	9	0.099 ¹
Age free of Seizures	(3d-3m) (8h-6m)				(8h-2d) (2d-6m)									
	3.5m	2.5	12 ¹	0.530*	NA					2m	3m	/	4	0.157 ¹
Mutation type	(10d-5m) (2m-5m)										(3m-5m)			
	Missense mutation	4	9	13	0.029 ²	4	5	9	9	1	3	5	9	NA
Others	5	0	5		0	0	0	0	0	0	0	0	0	
Mutation origin	<i>De novo</i>	3	8	11 ²	0.026 ²	3	5	8 ²	8 ²	1	2	5	8 ²	NA
	Parental	4	0	4		0	0	0	0	0	0	0	0	
Mutation position of gene	From Exon1 to Exon17	8	9	17 ³	0.388 ²	4	5	9	9	0.714 ²	1	3	5	9
Mutation position of protein	DEE-related Hot Spot	2	5	7 ³	0.335 ²		NA	NA						
Others	6	4	10 ³											
Mutation position of protein	Regions sensitive to ASMs	6	8	14 ³	0.576 ²	4	4	8	8	1	3	4	8	0.369 ²
	Others	2	1	3 ³		0	1	1	1	0	0	1	1	
EEG	HS or/and SB	0	9	9	0.000 ²	4	5	9	9	NA	1	3	5	9
	no	8	0	8 ¹		0	0	0	0	0	0	0	0	NA
SCBs	Yes	6	9	15	0.029 ²									
	No	3	0	3										
Effect of LEV	Effective	1	0	1	0.250 ²									
	Non-effective	1	6	7										
Effect of PB	Effective	5	3	8	0.182 ²									
	Non-effective	0	3	3										
Effect of VPA	Effective	3	6	9	0.523 ²									
	Non-effective	2	1	3										
Effect of NZP	Effective	0	3	3	0.250 ²									
	Non-effective	1	0	1										

T: total number, Neurodevelop.: neurodevelopmental outcomes, NA: not applicable, ¹: analyzed with Fisher Exact probability test, ²: analyzed with Mann Whitney U test, ³: analyzed with Kruskal-Wallis H test, ⁴: The age of onset and seizure free of PB was unclear, ⁵: the mutation origin of three patients was unclear, ⁶: the copy number variation was not included, ⁷: SCB was not used in three SeLE patients, ⁸: The age of one patient with SCB was unclear, SCB: Sodium channel blockers, SeLE: self-limited epilepsy, DEE: developmental and epileptic encephalopathy, HS: hypsarrhythmia, SB: suppression burst, ASMs: anti-seizure medications, d: days old, m: months old, h: hours old.

Table II. Continued.

Items	Phenotype		Seizure offset in				Neurodevelop. In DEE			T	P	
	SeLE	DEE	T	p	DEE		Mild	Moderate	Profound			
					Yes	No						
Effect of SCBs	6	9	15 ⁴	NA ⁴	4	5	9	1	3	5	9	NA
	0	0	0		0	0	0	0	0	0	0	0
Age with SCBs	5	9	14 ⁵	0.242 ⁴	/	/	9	/	/	/	9	0.188 ¹
Seizure Free	9	4	13	0.029 ⁴	NA	NA		1	3	0	4	0.008 ⁴
In Seizures	0	5	5					0	0	5	5	
Hypertonia	0	9	9	0.000 ⁴	NA	NA					NA	
	9	0	9									
Total	9	9	18		4	5	9	1	3	5	9	

T: total number, Neurodevelopmental outcomes, NA: not applicable, ¹: analyzed with Fisher Exact probability test, ²: analyzed with Mann Whitney U test, ³: analyzed with Kruskal-Wallis H test, ⁴: The age of onset and seizure free of Pt8 was unclear, ⁵: the mutation origin of three patients was unclear, ⁶: the copy number variation was not included, ⁷: SCB was not used in three SeLE patients, ⁸: The age of one patient with SCB was unclear, SCB: Sodium channel blockers, SeLE: self-limited epilepsy, DEE: developmental and epileptic encephalopathy, HS: hypsarrhythmia, SB: suppression burst, ASMs: anti-seizure medications, d: days old, m: months old, h: hours old.

were ineffective in this study. At their last visit, four patients (4/9) had been in seizure-free status since two to five months following birth, with a median of 3+ months. No statistical difference in effectiveness of SCBs, phenobarbital (p=0.182), valproic acid (p=0.523), levetiracetam (p=0.250), or nitrazepam (p=0.250) was found between SeLE and DEE. In 14 patients with SCBs, the age of starting SCBs was not associated with seizure offset (p=0.242), which was also found in nine DEE patients (p=0.072). In addition, age of onset (p=0.081) did not affect the seizure prognosis in DEE patients. In the follow-up study of nine SeLE patients and four relatives, seizures recurred in three (23.1%, 3/13, Pt3, Pt8 and Pt1's father).

ID/DD in nine DEE was unremitting. We found that seizure-free patients had better cognitive and movement ability than the patients with poorly controlled seizures (p=0.008). However, age of onset (p=0.099), seizure-free age (p=0.157), age with SCBs (p=0.188), did not affect their developmental outcome. Unfortunately, we failed to perform Gesell Developmental Scale tests. In addition, hypertonia was observed in all nine DEE patients.

Genetic analysis

Genetic analysis with next-generation sequencing was performed in 18 patients, and all of them were genetically diagnosed with seventeen single-nucleotide variations (SNV) and one copy number variant (Table I). Apart from three patients without all their parents' blood samples, eleven mutations were confirmed to be *de novo*, including eight in DEE patients (100%, 8/8), and three SeLE mutations were inherited from symptomatic parents. Pt9 inherited the mutation from her asymptomatic father.

According to the ACMG recommendations on SNV¹⁴, three variations (Pt2, Pt6, Pt8, 17.6%, 3/17) were pathogenic, eleven (64.7%, 11/17) were likely pathogenic, and the remaining three (Pt1, Pt4, Pt15, 17.6%, 3/17) were variations with uncertain significance (VUS). The three VUS

were not found in the normal control population and were reported in other research findings (Supplementary Table). The amino acids in the three sites (p.Val132Met, p.Leu640Trpfs*1 and p.Gly290Ser) were conserved in different species (<http://genome.ucsc.edu/>). Two of them (p.Val132Met and p.Gly290Ser) led to changes in amino acid properties. The wild-type amino acids were aliphatic and converted into sulfur-containing (p.Val132Met) or hydroxy amino acids (p.Gly290Ser). p.Leu640 was located in the last exon of *KCNQ2*, which might escape nonsense-mediated mRNA decay, however, segregation analysis revealed that the symptomatic members in the family (the proband and her mother) carried the variant whereas the healthy counterparts had the wild-type allele. Therefore, we considered those three variations to be clinically pathological, and all the patients with SNV were genetically diagnosed.

Among the SNVs, twelve missense mutations (70.6%, 12/17), one nonsense mutation (5.9%, 1/17), two small deletion mutations (11.8%, 2/17), one splice-site mutation (5.9%, 1/17), and one start-codon mutation (5.9%, 1/17) were detected, of which three (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G) were novel. SNVs were distributed in the exon 1-7, exon15 and exon 17. All nine DEE patients had missense mutations, and SeLE patients had missense mutation and other mutation types. There were differences in both mutation types ($p=0.029$) and mutation origin ($p=0.026$) between DEE and SeLE. We found that 35.3% (6/17) SNVs were located in C-terminal regions (Fig. 1), 23.5% (4/17) in the pore loop between S5 and S6, 11.8% (2/17) in S2, 11.8% (2/17) in the extracellular domain between S1 and S2, and 5.9% (1/17) in the N-terminal regions, S1, S4, and the intracellular domain between S4 and S5, respectively. Five DEE mutations and two SeLE mutations were located in S4, PD, and helices A. Fisher's precision probability test indicated no statistically significant difference in distribution between the DEE and SeLE in the *KCNQ2* gene ($p=0.388$), the DEE hot regions ($p=0.335$) or the

regions sensitive to ASMs ($p=0.576$). Neither mutation position in the gene nor the protein determined the seizure offset (the gene, $p=0.714$, the protein $p=1$), or ID/DD outcome (the gene, $p=0.571$, the protein $p=0.369$).

A novel copy number variation of 20q13.33del (chr20:61974641-62324656) containing fourteen genes including *KCNQ2*, *CHRNA4* (OMIM*118504), *EEF1A2* (OMIM*602959) and *RTEL1* (OMIM*608833) was found in Pt7. *KCNQ2* was an established haploinsufficiency gene, and the clinical features of Pt7, such as seizure type, EEG feature and response to oxcarbazepine was consistent with the features of *KCNQ2* related disease. It was considered pathological according to ACMG guidelines in copy number variation analysis.¹⁵

Review of previous patients

Thirteen kinds of mutations in fourteen patients in this study were reported (Supplementary Table). Only each of eight mutations resulted in SeLN (c.394G>A, c.1918delC) or DEE (c.1678C>T, c.683A>G, c.833T>C, c.868G>A, c.638G>A, and c.830C>T). While patients with DEE mutations were diagnosed with different epileptic syndromes, six patients with c.1678C>T (75%, 6/8), two with c.833T>C (50%, 2/4), and two with c.638G>A (28.6%, 2/7) were diagnosed with Ohtahara syndrome, and two patients with c.638G>A (28.6%, 2/7) were diagnosed with West syndrome. Besides, for the first time, we diagnosed Ohtahara syndrome in patients with c.683A>G, c.868G>A and c.830C>T, and diagnosed West syndrome in patients with c.1678C>T, c.683A>G and c.833T>C. Seizure prognosis of patients with the same DEE mutation (c.1678C>T, or c.638G>A) was variable. Half of patients (3/6) with c.1678C>T and 66.7% (2/3) patients with c.638G>A became seizure-free. All the patients including Pt15 (3/3) with c.868G>A became seizure-free. Prognosis of previous patients with c.683A>G, c.833T>C and c.830C>T were not available.

Four mutations (30.8%, 4/13, c.1741C>T, c.1A>G, c.998G>A, c.365C>T) were detected in both SeLE

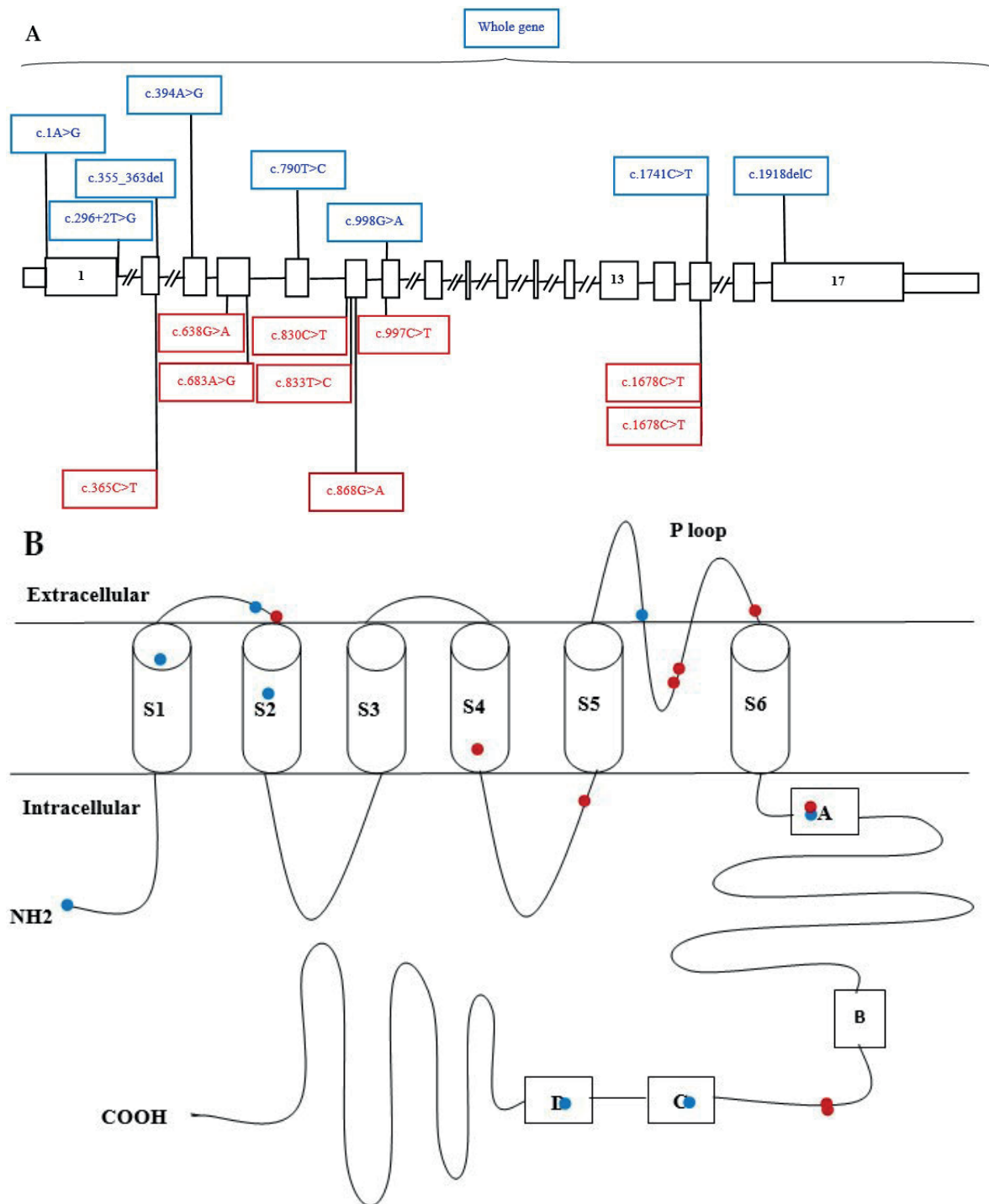


Fig. 1. (A) Mutations in *KCNQ2*. The middle of the figure is the *KCNQ2* structure. The black boxes represent exons, and the horizontal lines between the boxes are intron regions. The slightly shorter squares in exon 1 and exon 17 are non-coding sequences in exons. The size of exons and introns are based on the number their bases. Because the size of the figure is limited, we use slashes to represent the long introns. Mutations in blue were discovered in patients with self-limited epilepsy, and mutations in red were found in developmental and epileptic encephalopathy. **(B)** Mutations in *KCNQ2* protein. Mutations in blue were discovered in patients with self-limited epilepsy, and mutations in red were found in patients with developmental and epileptic encephalopathy. S: Segment.

and DEE patients. In this study, three patients (c.1741C>T, c.1A>G, c.998G>A) were SeLE, and one with c.365C>T were DEE. Pt8 in this study was the first SeLIE patient with c.1A>G, and Pt13 to be diagnosed with Ohtahara syndrome first in patients with c.365C>T. For patients in this and previous studies, we first found there was no statistical difference in seizure prognosis between DEE patients with mutations detected in both SeLE and DEE patients with mutations detected only in DEE ($p=1.0$). Clinical course of patients with c.997C>T (7.7%, 1/13) was not analyzed because the diagnosis of one previous patient was unclear.

Discussion

This study is a genetic and clinical analysis for epilepsy patients due to *KCNQ2* mutations, including the largest sample from South China. Electroclinical features in this study are consistent with previous reports^{4,7,10}, but we identified four novel *KCNQ2* mutations (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G, 20q13.33del) and discovered some distinct characteristics, which might result from the special genetic background of the patients or environmental factors in South China.

In this study, self-limited epilepsy (SeLE) (9/18) and DEE (9/18) accounted for half each, and the ratio of SeLNE (7/9) to SeLIE (2/9) was 7:2. Seizure and EEG/aEEG features, response to SCBs, seizure offset, and ID/DD outcome, mutations types were consistent with previous reports.^{7,11} In addition, we also found several distinct characteristics, including the difference in age of onset between SeLE and DEE, the benefit of seizure-free to ID/DD outcome. The age of onset of DEE was earlier than that of SeLE in this study. However, all the seizures started in infancy, we could not classify the patients by the age of onset alone. The age of onset, mutation location, and the age with SCBs did not determine the seizure or ID/DD offset. Opposite prognosis in Pt10 and Pt14 who had the same mutation suggested that other modifiers or environmental factors might

be involved in the pathogenesis of the disease. Previous studies have found several features of mutation distributions in *KCNQ2* protein that S4, ion pore domain (PD), helices A and B were discovered as four hot spot regions related to DEE, and mutations in patients who were responsive to ASMs mainly located in S2, PD, S4, S6, C-terminal region and extracellular region.⁴¹ However, no mutation distribution was similar between patients with DEE and SeLE or between patients with variable response to ASMs in this study. Besides, we found hypertonia in nine DEE patients, which has rarely been reported in previous reports.³⁵ Abnormal neuronal excitability after several gene mutations was found to be related to hypertonia⁴², which suggests that hypertonia and DEE might have a common pathogenic mechanism. Hypertonia was found in none of the SeLE patients, implying it to be an important symptom to distinguish DEE from SeLE. After treatment, nine SeLE patients in this study became seizures-free, and seizures in three family members disappeared spontaneously without treatment in infancy. This underlines the importance of identifying the etiology as early as possible to make the treatment decision and correctly predict the disease prognosis. Aggressive ASMs might not be required for SeLNE without frequent seizures and we may consider reducing or even stopping the ASMs earlier for SeLE to reduce the adverse reactions. It was reported that ketogenic diet was effective in *KCNQ2* related diseases⁴³, however, it had little effect in seizure control in this study. Different mutations and the special genetic background of South China might contribute to this, however more studies are needed to confirm this. In this study, 23.1% SeLE patients had recurrent seizure, which was higher than the proportion of 10-15% in previous reports⁷, reinforcing the idea that a long-term follow-up study is necessary for SeLE patients in South China.

In this study, we found four novel mutations (c.790T>C, c.355_363delGAGAAGAG, c.296+2T>G, 20q13.33del), expanding the

mutation spectrum of *KCNQ2*. Consistent with previous reports^{4,37}, *de novo* missense mutations often led to DEE. However, mutations in the three SeLE patients were *de novo*. Mosaicism in their parents might be a possibility.^{4,5} Mutations in Pt9 originating from an asymptomatic father may be explained by mosaicism in the father as well as an unreliable history. Genetic analysis for multiple tissues or RainDrop™ PCR of their parents would help to confirm this.

Variable phenotypes of 20q13.3 microdeletion syndrome, including seizures, brain malformation, ID/DD and psychological abnormalities, were reported.⁴⁴ In this study, we found a mild phenotype in Pt7 with 20q13.33 deletion, involving *KCNQ2*, *CHRNA4*, *EEF1A2*, *RTEL1* and other ten genes without identified diseases. Pt7's clinical features were different from epilepsy nocturnal frontal lobe (OMIM #600513) caused by *CHRNA4* mutations, DEE33 (OMIM #616409) resulting from *EEF1A2* mutations, and acute myeloid leukemia and dyskeratosis congenita due to *RTEL1* mutations. Furthermore, *CHRNA4* haploinsufficiency does not cause a disease and mutations in it are located in or close to the M2 region of the receptor and the gain-of-function effect is responsible for the disease.⁴⁵ Therefore, *KCNQ2* gene was considered the causative gene in Pt7, and we will also conduct a long follow-up study to observe whether other genes contribute to this phenotype.

In the analysis of clinical features of patients with thirteen kinds of reported mutations in this and previous reports, we found that patients were responsive to sodium channel blockers, and heterogeneity in epileptic syndrome and seizure prognosis was found in DEE mutations. Clinical heterogeneity might be the effect of other genetic modifier, and environmental factors. It was proposed that pre- or perinatal risk factors such as neonatal hypoxia and preeclampsia in pregnancy could amplify the pathophysiological impact of *KCNQ2* mutations.⁴⁶ Additional mutations in other genes or the involvement of other genetic variants that can further regulate the reduced

M-channel function may also play a role.²³ In addition, gender was once reported to be a factor in intrafamilial variability.⁴⁷ Pt13 with c.365C>T in our study suffered more serious symptoms than those in previously reported patients. We speculate that the thyroid dysfunction of his mother during pregnancy might have played a role. In addition, epilepsy syndromes of Pt8 (c.1A>G), Pt10 (c.1678C>T), Pt11 (c.683A>G), Pt12 (c.833T>C), Pt13 (c.365C>T), and Pt18 (c.830C>T) in this study has enriched the phenotype caused by the mutation they carried.

Generally, even though the mutations in *KCNQ2* were confirmed to lead to different phenotypes including SeLE and DEE, there were some overlaps in the mutation types, mutation origin, mutation distribution in the gene or the protein, and the positive response to SCBs, and different phenotypes were observed in patients with the same mutation even in the same family, illuminating that assessment of the impact of *KCNQ2* pathogenic variants is complicated, and a long-term follow-up study is necessary.

Unfortunately, the number of patients in this study was not large enough and therefore some of the results need to be confirmed with future research. We also did not conduct Gesell Developmental Scale test for most of the patients to evaluate their developmental outcome. Genetically, we failed to get the parents' DNA sample in three patients, which prevented us from determining the source of the mutation of the patients. In future studies, we will continue to conduct clinical and genetic analyses of more patients with *KCNQ2* gene mutations to make a greater contribution to the understanding of the disease.

Clinical and genetic analysis of eighteen patients from South China were conducted and this study identified four novel mutations and discovered some distinct features, which was enabled a deeper understanding of the clinical features of *KCNQ2*-related disease and the difference between SeLE and DEE in South China.

Supplementary materials

Supplementary materials for this article are available online at <https://doi.org/10.24953/turkjpediatr.2024.4593>

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Ethical approval

Our study was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center (No. 2019-40101). We have obtained informed consent from the patients' parents for the study.

Author contribution

The authors confirm contribution to the paper as follows: funding support, genetic data analysis, and drafting the first manuscript: BC; study design, data confirmation, manuscript reviewing and edition: BP; data collection and clinical analysis: YT, XL, HZ, and WC; aEEG analysis and drafting the EEG part of the manuscript: XW; statistical analysis and making tables and figures: HS. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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