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Clinical phenotypes of developmental and epileptic encephalopathy-related recurrent *KCNH5* missense variant p.R327H in Chinese children

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ABSTRACT

KCNH5 gene encodes for the voltage-gated potassium channel protein Kv10.2. Here, we investigated the clinical features of developmental and epileptic encephalopathy (DEE) in five Chinese pediatric patients with a missense mutation (p.R327H) in *KCNH5* gene. These patients had undergone video EEG to evaluate background features and epileptiform activity, as well as 3.0 T MRI scans for structural analysis and intelligence assessments using the Gesell Developmental Observation or Wechsler Intelligence Scale for Children. Seizure onset occurs between 4 and 10 months of age, with focal and generalized tonic-clonic seizures being common. Initial EEG findings showed multiple multifocal sharp waves, sharp slow waves or spike slow waves, and spike waves. Brain MRI revealed widened extracerebral space in only one patient. Mechanistically, the KCNH5 mutation disrupts the two hydrogen bonds between Arg327 and Asp304 residues, potentially altering the protein's structural stability and function. Almost 80 % of patients receiving add-on valproic acid (VPA) therapy experienced a reduction in epileptic seizure frequency. Altogether, this study presents the first Chinese cohort of pediatric DEE patients with the KCNH5 p.R327H mutation, highlighting focal seizures as the predominant seizure type and incomplete mutation penetrance. Add-on VPA therapy was likely effective in the early stages of DEE pathogenesis.

1. Introduction

Epilepsy is one of the most frequently diagnosed chronic neuropsychiatric disorders, affecting approximately 70 million people around the globe with an incidence rate of 0.5 % [1,2]. Several etiological factors, such as metabolic disorders, genetic disorders, abnormal immune activation, infections, and structural changes in the brain, have been identified as the pathological drivers of epileptic seizures. Disease-causing gene mutations have been discovered in several instances of epilepsy in humans. Interestingly, large-scale whole exome sequencing (WES) has revealed that individuals carrying pathologic mutations in the *KCNH5* gene manifest symptoms of neurodevelopmental disorders [3]. Reportedly, a missense mutation (p.R327H) in KCNH5 protein has been linked to developmental and epileptic encephalopathy (DEE) in pediatric patients of less than 1 year of age [3]. Despite these findings, mutant KCNH5 (p.R327H)-related phenotypes have not been investigated in detail. Because KCNH5 variants may promote diverse phenotypic expressions, phenotypic classification could be the most crucial part of epilepsy diagnosis and prognosis. Here, we report the analysis of cases of five unrelated patients with pathogenic KCNH5 (p.R327H) variant to delineate the phenotypic spectrum of mutant KCNH5 in the context of DEE pathogenesis. We also examined the major clinical features and outcomes of DEE-associated KCNH5 mutation in these patients.

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2. Materials and methods

This retrospective study was performed on five pediatric DEE patients carrying the KCNH5 R327H mutation who visited the Department of Neurology, Wuhan Children's Hospital, between March 2017 and September 2023. The study focused on certain phenotypic parameters such as age at seizure onset, electroencephalography (EEG) reports, brain magnetic resonance imaging (MRI) findings, seizure semiology, genetic inheritance evaluations, and DEE-specific syndromes. Epileptiform activities were evaluated using video EEG, whereas 3.0 T MRI examinations revealed the overall volume of the brain, ventricle sizes, callosal malformations, sulcal widening, alteration in cortical morphology, demyelination, and basal ganglia features. The patients' intelligence levels were assessed either by Gesell Developmental Observation (GDO) or Weschsler Intelligence Scale for Children (WISC). The WISC was validated for children over 6 years of age, and the GDO assessment was standardized for a wide age range of 4 weeks to 6 years in China. The seizure control group was defined as the absence of post-treatment seizures for a period three times longer than the maximum inter-seizure interval or one year, whichever was greater. DEE is characterized by developmental impairment caused by both the underlying etiology independent of epileptiform activity and epileptic encephalopathy. Frequent seizures refer to $\geq$ 4 episodes of epileptic seizures per month. Effective medications are considered clinically beneficial if they reduce 50 % in the frequency of seizures.

Peripheral blood (PB) samples were collected from five patients and their biological parents and analyzed for pathogenic gene mutations and gene variants using the trio WES platform, following the American College of Medical Genetics and Genomics (ACMG) recommended classification criteria. MutationTaster, SIFT, LRT, PolyPhen2, and FATHMM protein function prediction tools were used to predict mutation-associated altered protein functions. Validation and characterization of the parental origin of each gene mutation were further confirmed by Sanger sequencing and clinical feature analyses of all patients, excluding the *KCNH1* mutation. UCSF ChimeraX was employed for structural visualization of the mutant KCNH5 protein.

3. Results

3.1. Patient demographics

The five DEE patients' ages ranged from 2 years 2 months to 15 years 7 months. There were four male and one female subjects in the study population. Seizure onset was particularly observed in the age range of 4–10 months. The patients and their parents had no family relationships. There was no family history of DEE in these patient families. There were no pregnancy or congenital complications in these patients.

3.2. KCNH5 p.R327H-related DEE phenotypes and outcomes

All five patients presented with both focal (100 % of cases) and/or generalized tonic-clonic seizures in seizure semiology tests. EEG tests exhibited multiple multifocal sharp waves, sharp slow waves or spike slow waves, and spike waves. After treatment, only one patient's EEG features returned to normal, whereas there were no such improvements for the other four patients. Notably, only one subject presented with widened extracerebral space on MRI examination. MRI re-examinations of these patients revealed recovery of the cerebral symptoms. Valproic acid (VPA) add-on therapy was found to be effective for seizure control in four patients (80 %). These patients remained seizure-free until the final follow-up. The phenotypic features of all patients are summarized in Table 1.

3.3. Genotype-phenotype correlation analysis

We identified the de novo missense mutations in all patients, except one that was genetically inherited from the patient's father (Table 2). The KCNH5 p.R327H variant has been reported before in relation to DEE, however, we found that this variant had incomplete penetrance (Fig. 1). Patient #2 presented with a refractory epilepsy with severe developmental delay. Even treatments with multiple anti-seizure medications could not effectively manage his seizures. His father had the same mutation but had a normal phenotype and normal intelligence, suggesting that the KCNH5 p.R327H variant may have incomplete penetrance. Structural analysis of the KCNH5 mutant showed a loss of two hydrogen bonds between Arg327 and Asp304, which might also alter the conformational stability and function (Fig. 1).

Table 1
Summary of the phenotypic features in five patients.

P	Current age, Sex	Seizure onset age	Seizure semiology	Other Phenotype	Development retardation	EEG	MRI	ASMs tried	Effective ASMs	Seizure outcome
1	7y6m, M	7 *m*	GTCS, FS	No	Severe → Severe	Multifocal → Multifocal	N	LEV VPA	VPA	Controlled Free for 4y
2	15y7m, M	4 *m*	GTCS, FS	No	Severe → Severe	Multifocal → Multifocal	WG	LEV TPM OXC VPA LCM LTG CZP KD	NO	Uncontrolled
3	14y8m, M	7m20d	GTCS, FS	No	Moderate → Moderate	Multifocal → N	N	VPA	VPA	Controlled Free for 10y
4	4y, M	6 *m*	FS	No	Severe	Multifocal → Multifocal	N	LEV VPA	VPA	Controlled Free for 2y
5	2y2m, F	10 *m*	GTCS, FS	No	Severe	Multifocal	N	LEV TPM VPA	VPA	Controlled Free for 10 *m*

Abbreviation: P = patient; y = year/years; m = month; d = day; M = male; F = female; ASMs = anti-seizure medicines; FS = focal seizures; GTCS = generalized tonic-clonic seizure; Multifocal = multifocal sharp waves, spike waves, sharp slow waves or spike slow waves; LEV = levetiracetam; VPA = valproate acid; TPM = topiramate; OXC = oxcarbazepine; LCM = lacosamide; LTG = lamotrigine; CZP = clonazepam; KD = ketogenic diet; WG = widen gap in extracerebral space.

Table 2
Summary of the genetic findings in five patients.

P	Coordinates	Gene	Base change	Amino acid change	Predicted effect on protein	Zygosity	Inheritance	NM	Interpretation	Novel/ reported
1	Chr14:63 417,240	*KCNH5*	c.980G > A	p.R327H	Missense	Heterozygous	De novo	NM_139318	Pathogenic	R
2	Chr14:63 417,240	*KCNH5*	c.980G > A	p.R327H	Missense	Heterozygous	Father	NM_139318	Pathogenic	R
3	Chr14:63 417,240	*KCNH5*	c.980G > A	p.R327H	Missense	Heterozygous	De novo	NM_139318	Pathogenic	R
4	Chr14:63 417,240	*KCNH5*	c.980G > A	p.R327H	Missense	Heterozygous	De novo	NM_139318	Pathogenic	R
5	Chr14:63 417,240	*KCNH5*	c.980G > A	p.R327H	Missense	Heterozygous	De novo	NM_139318	Pathogenic	R

Abbreviation: P = patient; Chr = chromosome; R = reported, which means this mutation has been reported.

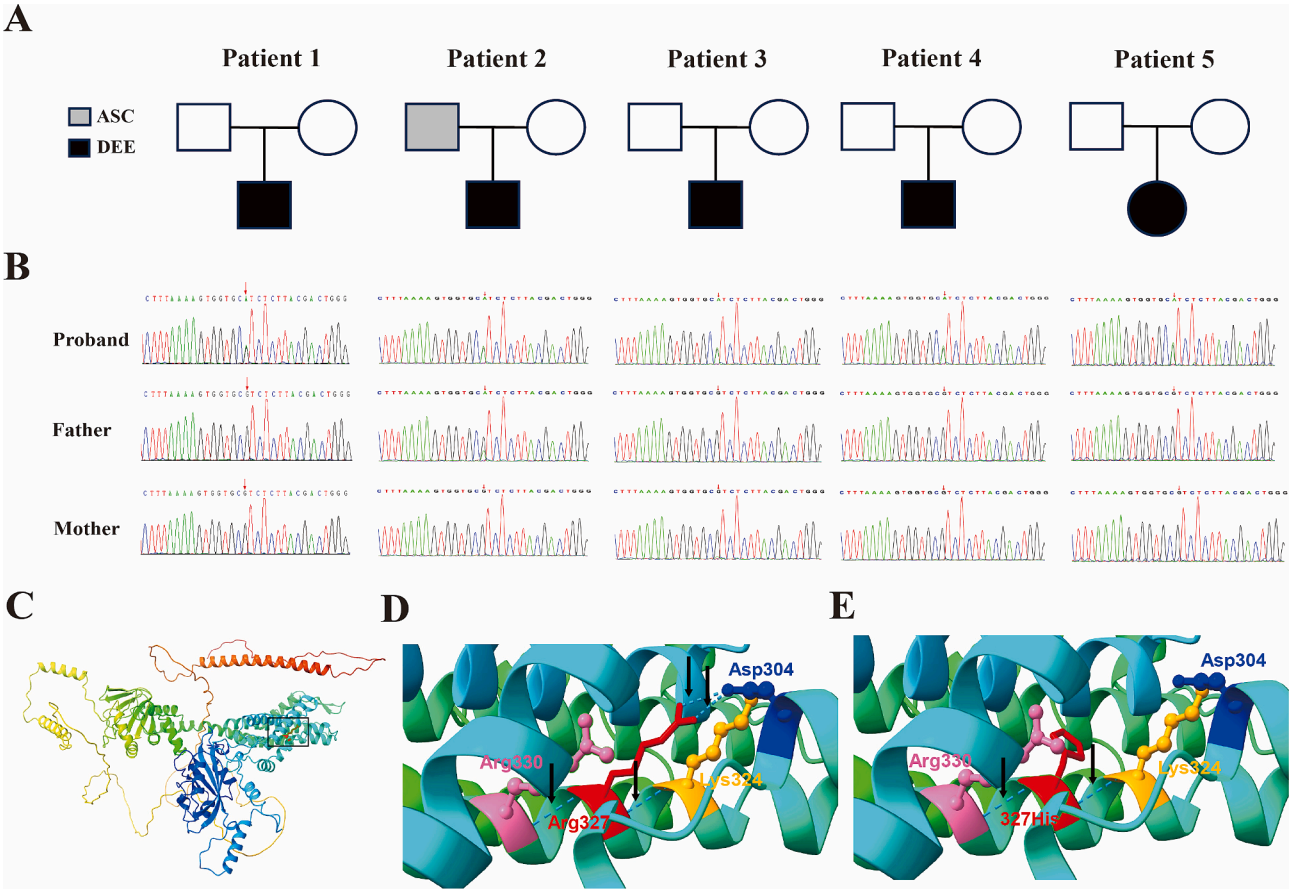

Fig. 1. Phenotypic spectrum in Chinese children with DEE-associated KCNH5 p.R327H mutation. (**A**) Phenotypic features in our 5 patients. Black indicates the proband with the DEE phenotype. Gray indicates the father of patient #2 has the ASC phenotype. (**B**) Sanger sequencing of *KCNH5* gene mutation. (**C**) 3D Panorama of KCNH5 protein. (**D**) Wild-type Arg327 (red) shows 4 hydrogen bonds (black arrow): Asp304 (blue), 2; Lys324 (orange), 1; Arg330 (hot pink). (**E**) Mutant 327His (red), 2 hydrogen bonds (black arrow): Lys324 (orange), 1; Arg330 (hot pink). Abbreviation: DEE = developmental and epileptic encephalopathy. ASC = asymptomatic carrier. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

In this study, we identified clinical phenotypes of the DEE-associated recurrent missense variant KCNH5 p.R327H in Chinese pediatric patients.

The *KCNH5* gene product Kv10.2 is primarily expressed in neurons of the olfactory bulb, cortex, thalamus, and hippocampus but not in the striatum and pituitary gland. The Kv10.2 expression is detected in the dendritic and axonal compartments of hippocampal neurons, where it regulates membrane excitability [4]. Increased activity of *KCNH* channels leads to decreased presynaptic and increased postsynaptic activities through homeostasis plasticity, suggesting that *KCNH* gain-of-function (GOF) mutations may contribute to hyperexcitability, resulting in seizures [5]. The kainic acid (KA)-induced temporal lobe epilepsy (TLE) model has been exploited to uncover the role of the Kv10.2 protein in status epilepticus (SE). Stereotactic injection of lentiviral KCNH5 expression vector (LV-KCNH5) into the rat hippocampal (right dorsal) CA3 subregion within 24 h of seizure induction showed improvement in anxiety-like behavior in the KA + LV-KCNH5 group compared with the vector control-treated SE group, indicating an important functional role of Kv10.2 in epilepsy [6].

In 2013, the first case of a KCNH5 R327H mutation reported the

seizure onset at 6 months of age, and the patient presented with generalized tonic-clonic (GTC) and hemiclonic seizures. His EEG showed frequent multifocal spikes and almost continuous discharge during sleep, even when seizures were well controlled. Seizures were recurrent during the 13-year follow-up duration [7]. During that time, the *KCNH5* gene mutation was first shown to be associated with epilepsy. Notably, several *KCNH5* missense variants, including *KCNH5* p.K324E, p.R327H, p.R333H, p.I463t, p.T468P, p.F471S, and p.S321N, have been associated with neurodevelopmental and epilepsy phenotypes [3,8]. However, KCNH5 p.R327H-related phenotypes have not been thoroughly investigated to date. The clinical phenotypes of *KCNH5* mutation-related disorders are heterogeneous, ranging from mild developmental delay without seizures to autism, dyskinesia, and severe DEE [3,8]. Among the KCNH5 missense mutants, the p.R327H variant is recurrent and mainly linked to DEE pathogenesis.

In these Chinese pediatric patients with the p.R327H mutation, DEE was the major phenotype with seizure onset within 4–10 months of age. Seizure semiology included GTC and focal (100 % of cases) seizures. The EEG lacked specificity and mostly exhibited multiple multifocal discharges but the brain MRI was unremarkable. We found that four patients had satisfactory seizure control and the add-on VPA therapy was effective in the early stages of the disease. Therefore, we conclude that the early addition of VPA therapy may be effective for KCNH5 p.R327H DEE patients. Contrary to previous findings, we found that KCNH5 p.R327H had an incomplete penetrance.

The KCNH5 p.R327H mutation in the S4 transmembrane segment destroys the essential feature of the channel voltage sensor. Moreover, the R327H variant induced two major biophysical changes:(1) enhanced activation at higher hyperpolarization potentials, along with a decrease in voltage dependence; and (2) the conductance − voltage relationship shifted significantly to the left (−70 mV) [9]. These could be the basis for epilepsy onset. The *KCNH5* gene mutation disrupted two hydrogen bonds between Arg327 and Asp304 residues, which may affect be the structure–function relationship of the mutant protein. “Variable expression” and “incomplete penetrance” are two useful operational constructs to describe such heterogeneity in clinical practice. Another interpretation could be that the major impact on gene network function caused by the abnormal function of a single gene, especially for a smaller gene network. Thus, the emergent clinical phenotypes could be the result of pathological interactions between multiple genes in that network rather than the impact of a single mutated gene [10,11]. For example, the *SCN1A* variant may be an important contributor to Dravet syndrome but the phenotype may involve the malfunction of more than one gene. In addition, genomic resilience may improve some serious outcomes, such as premature mortality in this disease [12]. Moreover, a child with DEE inherited the pathogenic variant p.R327H from an unaffected father, suggesting that mosaicism could be a possible explanation for incomplete penetrance or phenotypic variability. Further investigations are required to confirm the impact of one or two mutated gene(s) on the overall gene expression networks in various diseases.

In summary, we report the first Chinese cohort of KCNH5 p.R327H-associated DEE and the incomplete penetrance of this KCNH5 variant. Add-on VPA therapy could be effective in such patients, especially in their early stages. However, this is a retrospective analysis with a limited sample size. In the future, national or global multicenter studies are still needed to accumulate more samples and further conduct randomized controlled trials (RCT) and other studies to demonstrate reliability and effectiveness of VPA therapy in KCNH5-DEE patients.

5. Conclusion

The first Chinese cohort of five pediatric DEE patients with KCNH5 p.R327H mutation reveals focal seizure as the most common clinical phenotype in these patients. Lack of specificity in EEG and occurrences of multiple multifocal discharges are also common phenomena in such cases. However, the brain MRI could be unremarkable and add-on VPA therapy may be a clinically effective treatment option for this disease.

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Ethical statement

The project ethics were approved by the Ethic Committees of Wuhan Children’s Hospital, Tongji Medical College, Huazhong University of Science & Technology (2022R093-F02). All the experimental protocol for involving humans was by guidelines of the national/international/institutional or Declaration of Helsinki.

Statement

During the preparation of this work, we did not use artificial intelligence (AI) technology to write analytical data, manuscripts, etc.

CRediT authorship contribution statement

Sheng Huang: Formal analysis, Data curation, Conceptualization. **Chunhui Hu:** Writing – original draft. **Min Zhong:** Formal analysis, Data curation, Conceptualization. **Qinrui Li:** Formal analysis, Data curation, Conceptualization. **Yuanyuan Dai:** Visualization, Validation, Supervision. **Jiehui Ma:** Visualization, Validation, Supervision. **Jiong Qin:** Visualization, Validation, Supervision. **Dan Sun:** Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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