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ORIGINAL ARTICLE

Novel *KCNJ16* variants identified in a Chinese patient with hypokalemic metabolic acidosis

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Abstract

Background: Biallelic pathogenic variants in the *KCNJ16* gene result in hypokalemic tubulopathy and deafness (HKTD) (MIM #619406), which is a rare autosomal recessive disease characterized by hypokalemic tubulopathy with renal salt wasting, disturbed acid–base homeostasis, and sensorineural deafness. Currently, nine individuals with HKTD have been reported, and seven pathogenic variants in *KCNJ16* have been revealed.

Methods: A 5-year-6-month-old Chinese female patient displayed hypokalemic metabolic acidosis, salt wasting, renin-angiotensin-aldosterone system (RAAS) activation, arrhythmia, myocardial damage, cardiogenic shock and secondary diffuse brain oedema. Trio-based whole-exome sequencing (WES) was applied to detect the genetic cause.

Results: Novel compound heterozygous variants, c.190A>C (p.Thr64Pro) and c.628C>G (p.His210Asp), in *KCNJ16* were detected in the patient, and these variants were inherited from the patient's mother and father, respectively. Then, we systematically reviewed the available clinical manifestations of individuals with HKTD. We found that HKTD patients are at risk of cardiogenic shock and secondary diffuse brain oedema, which urges clinicians to make early diagnoses with prompt treatments.

Conclusion: These findings expand the variant spectrum of *KCNJ16*, enrich the clinical characteristics of HKTD, and provide a solid base for the genetic counseling, diagnosis and treatment of this condition.

KEYWORDS

cardiogenic shock, hypokalemic metabolic acidosis, hypokalemic tubulopathy and deafness, *KCNJ16*

Jianxiong Chen and Youqing Fu contributed equally to this work.

1 | INTRODUCTION

Hypokalemic tubulopathy and deafness (HKTD) is an autosomal recessive genetic disease characterized by hypokalemic metabolic acidosis, salt wasting and sensorineural deafness (Schlingmann et al., 2021). HKTD is caused by biallelic pathogenic variants in *KCNJ16* (OMIM# 605722) (Schlingmann et al., 2021; Webb et al., 2021). *KCNJ16* includes 5 exons, and only exon 5 is coding. *KCNJ16* encodes the inwardly rectifying potassium (Kir) 5.1 channel protein consisting of 418 amino acids. Kir5.1 is a member of a subfamily of Kir channels and contains two common motifs of putative transmembrane domains [TM1 (Arg71-Ile95) and TM2 (Ala146-Ala167)] connected with an extracellular pore-forming region [H5 (Ser117-Gln129)], cytoplasmic amino (NH2)-terminal domain (Met1-Trp70) and carboxy (COOH)-terminal domain (Ala168-Met418) (Zhang et al., 2020). Kir5.1 is expressed in the kidney, thyroid, pancreas and brain (Derst et al., 2001; Liu et al., 2000). Kir5.1 alone can form a homotetramer and can form heterotetramers with Kir4.1 (encoded by *KCNJ10*) or Kir4.2 (encoded by *KCNJ15*). Kir5.1 cannot form functional homomeric channels, but Kir5.1/Kir4.1 heteromers form the predominant inwardly rectifying potassium channel in the basolateral membranes of cells lining the distal convoluted tubule (DCT) and cortical collecting duct (CCD) (D'Adamo et al., 2011; Lourdel et al., 2002; Palygin et al., 2018; Tanemoto et al., 2000; Zhang et al., 2020). Kir5.1/Kir4.1 channels are considered to modify sodium delivery to downstream tubular segments to adjust potassium and proton secretion by sensing plasma potassium and intracellular pH with the resulting adjustment of the activity of the apical sodium chloride cotransporter (NCC) (Wang, 2016). HKTD is very rare; currently, only 9 HKTD patients and 7 pathogenic variants in *KCNJ16* have been reported (Schlingmann et al., 2021; Webb et al., 2021). Therefore, it is necessary to report more cases to enrich our knowledge of the clinical characteristics of this disorder and expand the variant spectrum of *KCNJ16*. Here, we identified two novel *KCNJ16* variants in a 5-year-6-month-old Chinese patient who presented strikingly similar phenotypes to those of HKTD and systematically reviewed the clinical characteristics of HKTD patients.

2 | MATERIALS AND METHODS

2.1 | Trio-based whole-exome sequencing (WES)

Trio-based whole-exome sequencing was performed for the family members to detect causal variants. Sequencing was performed with an Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA). Suspected variants were confirmed by Sanger sequencing. The pathogenicity of the sequence variants was assessed according to ACMG/AMP guidelines (Richards et al., 2015).

3 | RESULTS

3.1 | Case report

The proband was a 5-year-6-month-old female with a nonconsanguineous union. She was born at full term by vaginal delivery. She had completely normal cognitive, physical and psychomotor development. At 5 years and 6 months of age, she suffered from fever, vomiting, progressive fatigue, polydipsia and polyuria. She was referred to the intensive care unit for her weakness, cyanosis and sudden unconsciousness. Then, cardiopulmonary resuscitation was immediately performed for the patient due to a rapidly decreased heart rate to cardiac arrest. This led to cardiogenic shock, as follows: hypotension (69/33 mmHg, reference range: 90 ~ 140/60 ~ 90 mmHg), pale skin, purple mucosa and lips and cold, clammy skin. Laboratory tests showed metabolic acidosis and electrolyte disturbances, including reduced blood pH level (7.3, reference range: 7.35–7.45), $HCO3^{-}$ level (19.8 mmol/L, reference range: 22–27 mmol/L), PCO2 level (32 mmHg, reference range: 35–45 mmHg), and base excess (BE) level (−4.3 mmol/L, reference range: 0 ± 2.3 mmol/L), severe hypokalemia (1.18 mmol/L, reference range: 3.5–5.5 mmol/L), hyponatremia (124.9 mmol/L, reference range: 136–145 mmol/L), hypochloremia (89.9 mmol/L, reference range: 98–108 mmol/L), hypocalcemia (1.79 mmol/L, reference range: 2.25–2.75 mmol/L) and hypophosphatemia (0.75 mmol/L, reference range: 1.1–1.3 mmol/L). Laboratory tests also showed signs of myocardial injury, including elevated creatine kinase (CK) (698 mmol/L, reference range: 0–170 mmol/L), CK-MB (68 mmol/L, reference range: 0–25 mmol/L), cTnT (1.91 ng/mL, reference range: 0–0.78 ng/mL), MYO (500 ng/mL, reference range: 0–110 ng/mL) and LDH (262 U/L, reference range: 120–250 U/L). In addition, laboratory tests indicated RAAS activation, including elevated serum random aldosterone (1484 pg/mL, reference range: 30–160 pg/mL) and renin activity (4.96 ng/mL.h, reference range: 0.15–2.33 ng/mL.h). Then, Klebsiella pneumoniae was identified by bacterial culture. Brain MRI was performed at this time and showed diffuse oedema in the bilateral cerebral hemispheres, basal ganglia and cerebellar hemispheres. The patient displayed slightly altered mental status and gait abnormalities. Electrocardiogram showed malignant arrhythmia. Cardiac and renal ultrasound was normal. In addition, brainstem-evoked response audiometry revealed unimpaired hearing. The patient was given active

treatment, including electrocardiographic monitoring, ventilator-assisted ventilation, cardiopulmonary resuscitation, intravenous injection of epinephrine, cefotaxime sulbactam anti-infection, potassium chloride supplement, ganglioside ester for nerve nutrition, and dalteparin for the prevention of venous thrombosis. The patient recovered well and remained asymptomatic.

3.2 | Genetic analysis

Novel compound heterozygous variants, c.190A>C (p.Thr64Pro) and c.628C>G (p.His210Asp), in *KCNJ16*, were revealed in the proband, which were inherited from the mother and father, respectively (Figure 1a). The Thr64 and His210 residues are highly conserved among different species (Figure 1b). The variant (c.190A>C, p.Thr64Pro) is not present in the 1000 Genomes Project or the Genome Aggregation Database (PM2), and the variant (c.191C>T, p.Thr64Ile) affecting the Thr64 residue has been report to be pathogenic in the literature (Schlingmann et al., 2021) (PM5). The allele frequency of the variant c.628C>G is 0.00002408 in the Genome Aggregation Database (PM2). Both variants were predicted to have a deleterious effect on the protein product by multiple in silico prediction tools (MutationTaster, SIFT and PolyPhen-2) (PP3). In

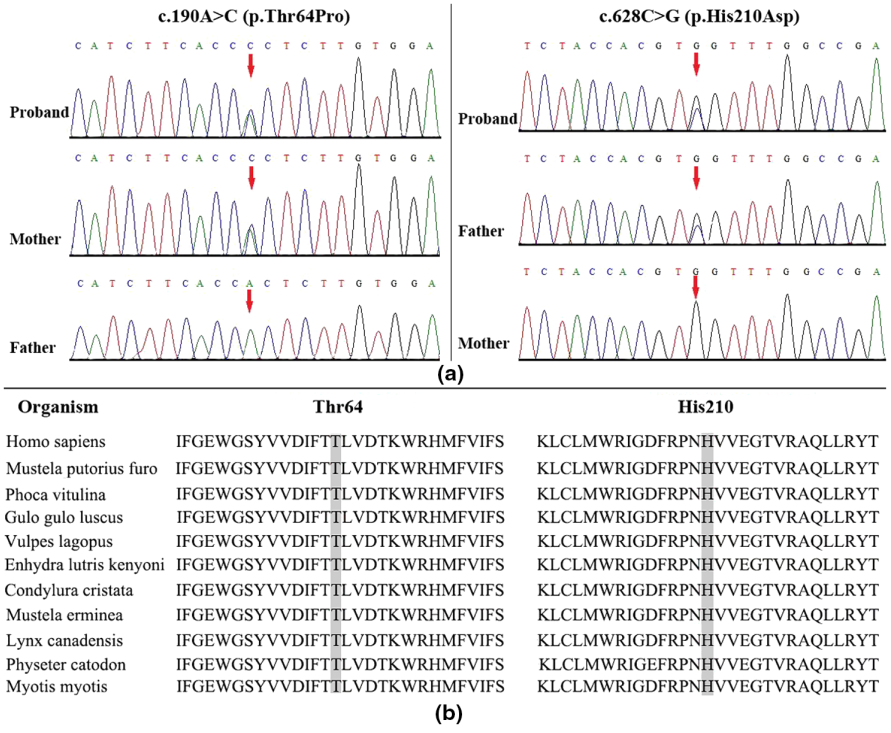

FIGURE 1 Variant identification by Sanger sequencing. Compound heterozygous variants c.190A>C and c.628C>G in *KCNJ16* were detected in the patient and her asymptomatic parents. The red arrow indicates the variant site (a). Location of patient's *KCNJ16* missense variants (c.190A>C, p.Thr64Pro and c.628C>G, p.His210Asp) on a highly conserved multi-species alignment of KCNJ16 protein sequence; shaded area stands for the residue Thr64 and His210 (b).

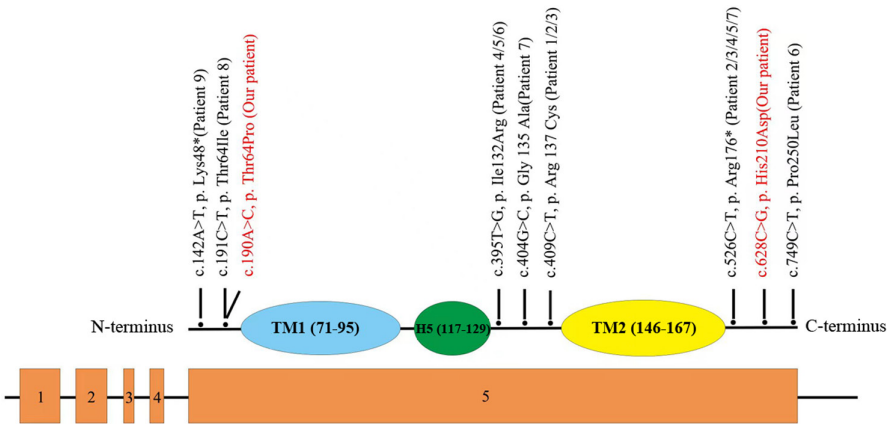

FIGURE 2 Schematic representation of *KCNJ16* variants identified to date. The structure of *KCNJ16* contained 5 exons (organge rectangles), and introns (black horizontal line); lower side: the KCNJ16 protein domains: cytoplasmic amino (NH2)-terminal domain; TM1 domain; H5 domain; TM2 domain; cytoplasmic amino carboxy (COOH)-terminal domain. The localization of variants and substitutions identified is signed with dots. Black: Variants identified in the literature; Red: Novel variants detected in this study.

TABLE 1 Overview of variants and phenotypes observed in patients with *KCNJ16*-related hypokalemic tubulopathy and deafness.

	Patient 1 Schlingmann et al. (2021)	Patient 2 Schlingmann et al. (2021)	Patient 3 (patient 2's brother) Schlingmann et al. (2021)	Patient 4 Schlingmann et al. (2021)	Patient 5 Schlingmann et al. (2021)
Sex	F	F	M	M	F
Consanguinity	+	–	–	–	–
Age at onset	5 days	14 months	18 months	5 years old	4 years old
Predisposing factors	–	Gastroenteritis	–	Fever	–
Variants	c.409C>T, p.R137C (homozygous)	c.409C>T, p.R137C c.526C>T, p.R176*	c.409C>T, p.R137C c.526C>T, p.R176*	c. 395T>G, p.I132R c.526C>T, p.R176*	c. 395T>G, p.I132R c.526C>T, p.R176*
Clinical findings					
Hypokalemia (normal range: 3.5–5.5 mmol/L)	+ (1.8)	+ (1.8)	+ (3.0)	+ (1.2)	+ (2.7)
Acidosis	+	+	+	+	+
Sensorineural deafness	+ (5 days)	+ (8 years)	+ (18 months)	+	+ (4.5 years)
Renal salt wasting	+	+	–	–	+
Hyper-reninism	+	+	NA	+	+
Hyperaldosteronism	+	+	NA	+	–
Hypochloremia	+	–	–	+	+
Hyponatremia	–	–	–	+	–
Hypocalcemia	–	–	NA	–	–
Constipation	–	–	–	–	+
Polyuria	+	–	–	–	–
Others	Hypomagnesemia	–	–	–	–

Abbreviations: –, absent; +, present; F, female; M, male; NA, not available.

addition, the patient's phenotypes were highly consistent with HKTD (PP4), and trio-based WES also excluded other possible known genetic causes. The variant c.628C>G is in trans with the c.190A>C (PM3). Thus, both variants were assessed to be likely pathogenic and responsible for the clinical phenotypes of our patient according to ACMG/AMP criteria (Richards et al., 2015).

4 | DISCUSSION

Homozygous or compound heterozygous variants in *KCNJ16* cause HKTD. Currently, only 7 pathogenic variants in *KCNJ16* have been identified, and 9 patients with HKTD have been reported (Schlingmann et al., 2021; Webb et al., 2021). Here, we report a 5-year-6-month-old female patient who displayed hypokalemic metabolic acidosis, salt wasting, hyponatremia, RAAS activation, polyuria, arrhythmia, myocardial damage, cardiogenic shock and secondary diffuse brain oedema, which were triggered by minor illness. Two novel missense variants (c.190A>C, p.Thr64Pro and c.628C>G, p.His210Asp) in *KCNJ16* were revealed in the patient. Our patient's symptoms were strikingly similar to those of HKTD. Thus, both variants were likely to be responsible for our patient's clinical manifestations. These novel variants expand the *KCNJ16* variant spectrum and could help in the molecular diagnosis of HKTD. Next, we reviewed all *KCNJ16* variants. A total of 9 pathogenic variants, including the two novel variants detected in this study, were identified (Schlingmann et al., 2021; Webb et al., 2021). *KCNJ16* nonsense variants and missense variants accounted for 22.2% (2/9) and 77.8% (7/9) of variants, respectively. Interestingly, none of the variants were located in the known domains (TM1, TM2 and H5 domain). The mutation hotspots seem to focus on the following variants: I132R, R137C and R176* (Figure 2). Therefore, more cases are required to further expand the variant spectrum of *KCNJ16*.

We analyzed the clinical phenotypes of all individuals with HKTD to comprehensively profile the condition (Table 1). The main clinical features included hypokalemia (10/10), metabolic acidosis (9/10), sensorineural hearing

Patient 6 Schlingmann et al. (2021)	Patient 7 Schlingmann et al. (2021)	Patient 8 Schlingmann et al. (2021)	Patient 9 Webb et al. (2021)	Our patient	Incidence of clinical features
F	F	F	F	F	
–	–	+	+	–	
26 years old	16 years old	22 years old	21 months	5.5 years old	
–	–	–	Fever	Fever	
c. 395T>G, p.I132R c.749C>T, p.P250L	c. 404G>C, p.G135A c.526C>T, p.R176*	c.191C>T, p.T64I (homozygous)	c.142A>T, p.K48* (homozygous)	c. 190A>C, p.T64P c.628C>G, p.H210D	
+ (2.5)	+ (1.5)	+ (2.8)	+ (3.2)	+ (1.18)	10/10
+	+	–	+	+	9/10
+	+ (5 years)	+ (14 years)	NA	–	8/10
+	+	+	–	+	7/10
+	+	+	–	+	8/10
+	–	+	–	+	6/10
–	–	–	–	+	4/10
–	–	NA	+	+	3/10
+	+	–	–	+	3/10
–	+	–	–	–	2/10
–	–	–	–	+	2/10
Palpitation and loss of consciousness	Rhabdomyolysis; elevated CK	Alkalosis; dyspnea	Hypoparathyroidism; diffuse intracranial calcifications; hyperchloremia	Diffuse brain oedema; adverse cardiac events; elevated CK; hypophosphatemia	

loss (8/10), hyperreninism (8/10), renal salt wasting (7/10), hyperaldosteronism (6/10), hypochloremia (4/10), hyponatremia (3/10), hypocalcemia (3/10), constipation (2/10) and polyuria (2/10). As reported, high-frequency sensorineural deafness is a highly common feature in HKTD. Uniformly, sensorineural hearing impairment was diagnosed in childhood or adolescence. To date, our female patient did not show this feature, which deserves further follow-up. Of all the patients, only our patient displayed obvious hypotension, arrhythmia, myocardial injury, recurrent cardiac arrests (three times) and even cardiogenic shock, although the RAAS system was activated. Then, she displayed secondary diffuse brain oedema and transient alterations in mental status and abnormal gait. However, she recovered well from active and effective treatments, and no sequelae were observed. Next, we noticed that Patient 6 previously reported also displayed palpitations and loss of consciousness (Schlingmann et al., 2021). So it should be alerted that HKTD patients with hypokalemia, salt wasting and metabolic acidosis are at risk of cardiogenic shock with its potential complications, such as brain oedema, respiratory failure, myocardial infarction, cerebrovascular accidents, intestinal ischaemia and so on. Thus, clinicians should be mindful of this condition, and early diagnosis with prompt treatments is urgently needed.

In conclusion, we identified novel compound heterozygous variants in *KCNJ16* in a Chinese patient with HKTD, which expands the variant spectrum of *KCNJ16*. Next, we systematically reviewed the clinical features of HKTD and found that HKTD patients are at risk of cardiogenic shock with its potential complications, which requires an urgent diagnosis and treatments. These findings will enrich our knowledge of the clinical characteristics, clinical management and genetic counseling of HKTD.

AUTHOR CONTRIBUTIONS

Jianxiong Chen and Youqing Fu drafted the first version of the manuscript. Haiming Yuan was responsible for the design of the project and polished the versions of the manuscript. Xinlong Zhou, Qingming Wang, Cong Li and Yan Sun were responsible for data analysis, clinical assessment and the design of the project. YS provided financial

support. All authors have read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Dongguan Maternal and Child Health Care Hospital. Written informed consent was obtained from the families.

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