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ORIGINAL ARTICLE

Clinical Features and Unusual Heterozygous Mutations in Patients with Renal Hypokalemia

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SUMMARY

Background: **Renal hypokalemia is associated with mutation. This study aimed to investigate the clinical features and pathogenic mutations in patients with renal hypokalemia.**
Methods: **The patients with hypokalemia were enrolled, and the renal function, thyroid function, renin-aldosterone system, urinary potassium excretion, and exome sequencing were performed. The correlation between the clinical phenotypes and causative genes was assessed.**
Results: **Five patients with hypokalemia were enrolled and diagnosed as tubular hypokalemia. The patients with common clinical manifestations were difficult to differentiate based on atypical laboratory findings. The results of the genetic analysis were as follows: both patient 1 and patient 2 were heterozygous for the c.C625T mutation of the *KCNJ1* gene, which is responsible for Bartter syndrome. Patient 3 was heterozygous for the c.G298A mutation of the *ATP6V1B1* gene, which is responsible for renal tubular acidosis. Patient 4 had a compound heterozygous mutation of c.G893A of the *BSND* gene, responsible for Bartter syndrome, and c.1029+5G>A, the *ATP6V0A4* gene responsible for distal renal tubular acidosis. Patient 5 had Gitelman syndrome and carried the compound heterozygous mutations c.C1963T and c.G2029A of the *SLC12A3* gene. All the above loci were known heterozygous mutations.**
Conclusions: **The unusual heterozygous mutations were identified in five renal hypokalemia patients. Molecular diagnosis of tubular hypokalemia was conducive to accurate diagnosis and treatment.**
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INTRODUCTION

Hypokalemia is a series of diseases characterized by level of serum K^+ concentration < 3.5 mmol/L and is a common electrolyte disorder observed in the clinics. Hypokalemia is classified according to its etiology: insufficient potassium intake, excessive potassium excretion, and metastatic hypokalemia. Renal loss of potassium is defined as excessive loss of potassium through the kidneys, which is commonly observed in various renal diseases and endocrine diseases and as a drug-related side effect. Nephrogenic hypokalemia is associated with a wide range of diseases, insidious onset, and com-

plex mechanisms, and a considerable proportion of cases are associated with hereditary diseases, which require further genetic testing for definitive diagnosis [1,2].

Renal hypokalemia can be divided into two categories: the first category is characterized by abnormalities in the solute transport system of the distal tubule and/or collecting ducts, increased sodium reabsorption, and decreased potassium reabsorption; this category includes Liddle's syndrome, Bartter syndrome (BS), Gitelman syndrome (GS), anterograde hydrocorticosteroid overdose, Gordon's syndrome, and hypertension exacerbated by pregnancy. The second category is characterized by abnormalities in the synthesis of adrenal steroid hormones, which leads to abnormal activation of the hydrocorticosteroid receptor in the distal renal unit and an increase in sodium reabsorption in the distal tubule; this category includes familial hypercorticosteroidism, familial aldosteronism, congenital adrenocortical hyperplasia, and familial glucocorticoid resistance syndrome [2-5].

In this study, genetic testing was used to investigate the mutation in patients with renal hypokalemia, to clarify the diagnosis of patients with recurrent hypokalemia. Patients with clinically difficult hypokalemia were screened, their pathogenic gene mutations and clinical phenotypes were analyzed, and their pathogenic mechanisms were explored. The results could provide data support and a theoretical reference for molecular genetic mechanisms in these patients.

MATERIALS AND METHODS

Patients

The baseline information of patients with hypokalemia was obtained at admission through consultation, physical examination, and laboratory tests, including blood pressure, serum and urinary electrolyte profile, renal function, blood gas analysis, thyroid function, cortisol, renin-aldosterone, electrocardiogram, and adrenal computed tomography (CT). The clinical data are shown in Table 1. Patients with renal potassium loss, defined as the loss of excess potassium through the kidneys, were screened according to the etiology of hypokalemia. Diagnosis was made based on 24-hour urine potassium levels, and renal loss of potassium was diagnosed at a blood potassium level of < 3.5 mmol/L and a urine potassium level of >25 mmol/24 hours [3,5]. The study was approved by the medical Ethics Committee of the hospital, and the subjects provided written informed consent.

Genetic analysis

We commissioned Jingneng Biotechnology (Shanghai) Co., Ltd., to perform whole-exome sequencing of the patients using an Illumina HI4000. When pathogenic variants were detected, they were analyzed online by Mutation Taster, PolyPhen-2, SIFT, PROVEAN, and Human Splicing Finder. Then, we commissioned Beijing Zhongmei Taizhouhe Co., Ltd., to validate the results using Sanger sequencing. At the same time, we followed up with the patient's family to verify whether any members of the patient's family had the same mutation by Sanger sequencing. The pathogenicity of the mutations was evaluated according to the criteria and guidelines for pathogenic variants issued by the American Society for Medical Genetics and Genomics.

RESULTS

All five patients met the criteria for clinical diagnosis of renal potassium loss and exhibited signs and symptoms related to hypokalemia that were relieved by potassium or magnesium supplementation. Thyroid function, blood cortisol, and adrenal CT were normal. The results of gene sequencing and variant analysis were as follows: patient 1 (from Jiangsu) and patient 2 (from Guangxi) both carried the same mutation c.625C>T in the exon 2 of *KCNJ1* gene, which is responsible for BS; these patients lived 1,600 km away from each other but exhibited the same manifestations. Patient 3 had carried the heterozygous mutation c.G298G>A in the exon 4 of *ATP6V1B1* gene, which is responsible for renal tubular acidosis. Patient 4 exhibited low blood potassium and magnesium levels and increased urinary potassium excretion with suppression of the renin-aldosterone system and had a compound genotype: the heterozygous mutation c.893G>A in the exon 4 of *BSND* gene, responsible for BS, and the heterozygous mutation c.1029 +5G>A in exon 11 of the *ATP6V0A4* gene, responsible for distal renal tubular acidosis (dRTA). Patient 5 had a definite diagnosis of GS and carried the compound heterozygous mutations c.C1963C>T and c.2029G>A in the exon 16 of *SLC12A3* gene. The sequences are shown in Figure 1.

DISCUSSION

The present study revealed the pathogenic mechanism of renal potassium loss in five patients through a comprehensive evaluation and analysis of laboratory tests. Manifestations of hypokalemia caused by gene mutations are mostly inherited in a recessive manner, such as GS and BS, although a few are inherited in a dominant manner, such as Liddle syndrome. The clinical presentation, electrolyte profile, renin-aldosterone system, and acid-base balance often are not sufficient to confirm the diagnosis [3].

In this study, two patients (patients 1 and 2), separated by a substantial distance, both harbored the heterozygous mutation c.625C>T in *KCNJ1* gene, which demonstrated pathogenicity. Patient 3 carried the heterozygous mutation c.G298G>A in *ATP6V1B1* gene, which also demonstrated pathogenicity. Patient 4 harbored a compound mutation in the complex gene compound, which

Table 1. Baseline information and examination results of five patients with hypokalemia.

Patient	Gender (F/M)	Age (years)	SBP/DBP (mmHg)	Serum Na^+ (135 - 145 mM)	Serum K^+ (3.5 -5.3 mM)	Serum Mg^{2+} (0.75 -1.02mM)	Serum creatinine (41 - 81 μmol/L)	24-hour urine potassium (< 20.0 mM)	Plasma $HC03^-$ (18 - 23 mM)	Arterial pH
1	M	51	118/81	146.1	2.15	0.65	37.00	27.38	34.30	7.53
2	M	47	169/116	141.7	3.22	0.86	65.00	55.76	27.50	7.43
3	M	52	155/88	141.7	3.39	0.85	66.00	26.75	31.20	7.44
4	M	29	128/74	142.9	3.41	0.93	64.00	46.20	24.80	7.44
5	M	32	127/91	134.9	2.48	0.65	71.00	45.60	25.50	7.42

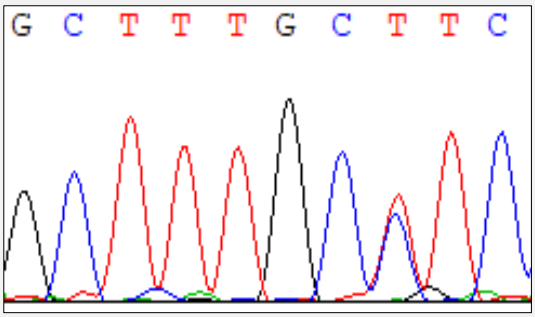
Renin standing (4.0 - 38.0 pg/mL)	Aldosterone standing (40.0 - 310.0 pg/mL)	Genotypes	Clinical manifestation
9.89	74.64	*KCNJ1*: exon2 c.C625T	Weakness with numbness in the limbs
5.94	114.54	*KCNJ1*: exon2 c.C625T	Weakness in all four limbs
1.27	132.09	*ATP6V1B1*: exon4 c.G298A	Weakness in all four limbs
2.47	94.10	*BSND*: exon4 c.G893A and *ATP6V0A4*: exon11 c.1029+5G>A	Weakness in all four limbs
7.20	199.07	*SLC12A3*: exon16 c.C1963T and c.G2029A	Weakness and unresponsiveness of the limbs

was an unexpected finding. Laboratory test results in patient 5 were consistent with the genetic diagnosis. Through molecular diagnosis, we can more accurately determine the etiology of the disease and provide more precise treatment strategies.

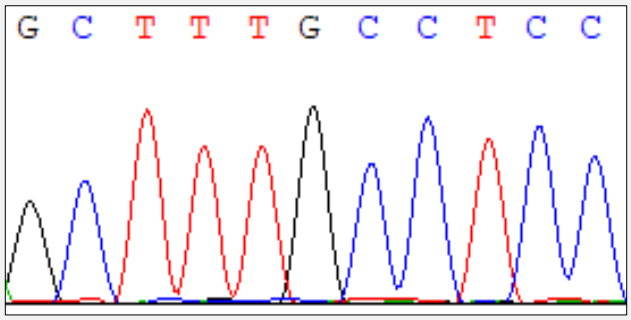
Currently, renal potassium loss is mainly diagnosed in the clinic by using 24-hour urine potassium, with the criteria of blood potassium concentration < 3.5 mmol/L and urine potassium concentration > 25 mmol/24 hours. This condition can be caused by renal disease, endocrine disease, and oral potassium-expelling drugs. The differential diagnosis is made by monitoring blood pressure and analyzing renin, aldosterone, and blood gas levels. Although patients 1 and 2 had milder clinical manifestations, both were equally morbid for single-gene heterozygous mutations. Both patient 1 and 2 carried the heterozygous mutation c.C625T in exon 2 of the *KCNJ1* gene responsible for BS. These patients live 1,600 km apart in China and are not related by blood, but both patients exhibited pathogenicity associated with the heterozygous mutation c.C625T in exon 2 of the *KCNJ1* gene. BS is an autosomal recessive disorder, and only pure and compound heterozygotes are pathogenic; however, both of these patients were heterozygous and clinically presented with mild hypokalemia, which is a valuable finding. The c.C625T mutation in this gene has been poorly studied, and our results enrich the genetic spectrum.

BS is an inherited renal tubular disease caused by mutations in genes encoding or regulating related transporter proteins in the thick segment of the ascending branch of the renal tubular medullary collaterals and the distal tubule [4]. BS is classified into types I-V. Type I BS is caused by a mutation in the sodium-potassium chloride cotransporter gene (*SLC12A1*), type II BS is caused by a mutation in *KCNJ1*, type III BS is caused by a mutation in *CLCNKB*, type IVa BS is caused by a mutation in *BSND*, and type IVb is caused by mutations in *CLCNKA* and *CLCNKB*. Both patient 1 and patient 2 in this study carried the heterozygous mutation c.C625T in exon 2 of the *KCNJ1* gene responsible for BS. Patient 4 was diagnosed with type IVa BS according to the gene mutation. Type IVa BS is caused by a common subunit defect in the barttin-encoding gene *BSND* (OMIM: 606412), which is located on chromosome 1p32 and consists of four exons. Barttin is distributed in the inner ear as well as in the renal tubules, so type IVa BS often occurs in combination with sensorineural deafness [6]. A total of 18 *BSND* gene variants have been found to be associated with type IVa BS, two of which are also associated with asymptomatic sensorineural deafness [7]. *BSND* encodes the membrane-integrating protein barttin, the β-subunit shared by the chloride reabsorption channels ClC-Ka and ClC-Kb. Barttin enhances the membrane localization and increases the conductivity of ClC-K. Mutations in this protein are responsible for hypokalemia, hypochloremia, metabolic alkalosis, hyperrenin-induced angiotensinemia, hyperaldosteronism, and normotension and are often combined with neurodeafness. The main manifestation is hypokalemia. The treatment of BS is based on symptomatic support and focuses on maintaining relatively normal serum electrolyte levels. In BS, hypokalemia can lead to significant complications, such as muscle weakness and cardiac arrhythmias; therefore, potassium ion supplementation should be an area of focus. Potassium supplementation, for which potassium chloride is preferred, can be combined with potassium-preserving diuretics, such as Spi-

Patient 1

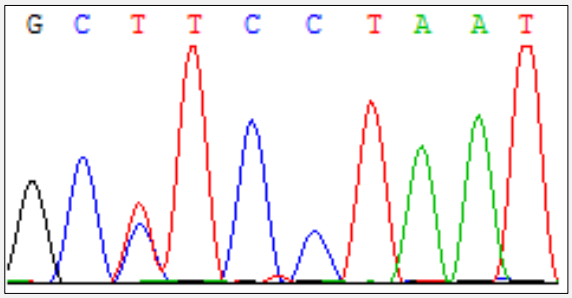


***KCNJ1*: exon2 c.C625T**

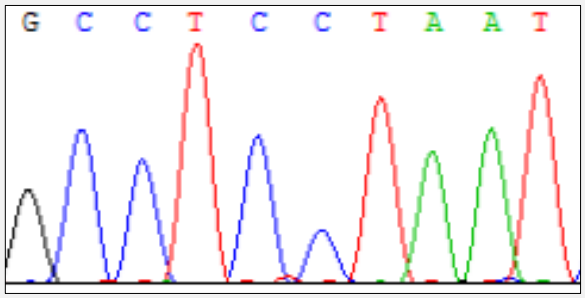


***KCNJ1*: exon2 c.C625**

Patient 2

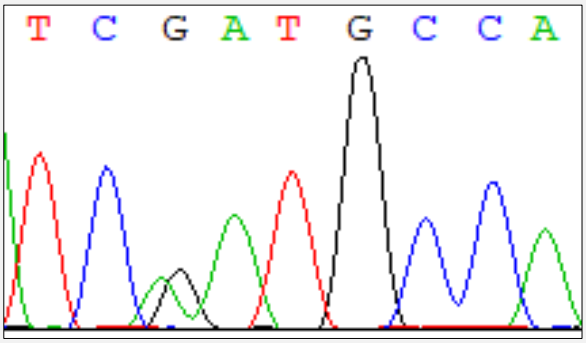


***KCNJ1*: exon2 c.C625T**

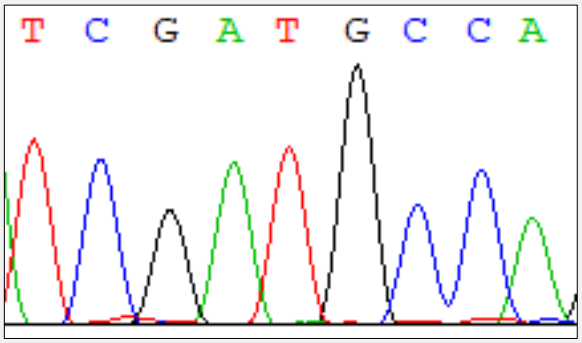


***KCNJ1*: exon2 c.C625**

Patient 3

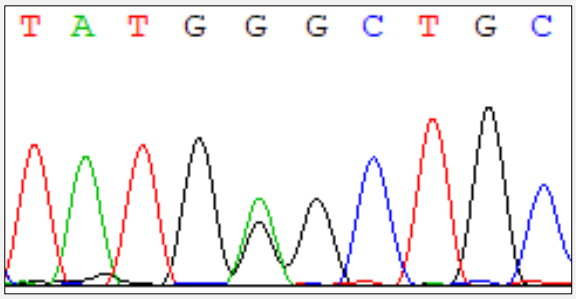


***ATP6V1B1*: exon4 c.G298A**

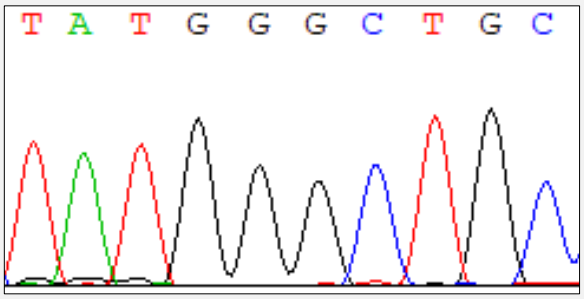


***ATP6V1B1*: exon4 c.G298**

Patient 4



***BSND*: exon4 c.G893A**



***BSND*: exon4 c.G893**

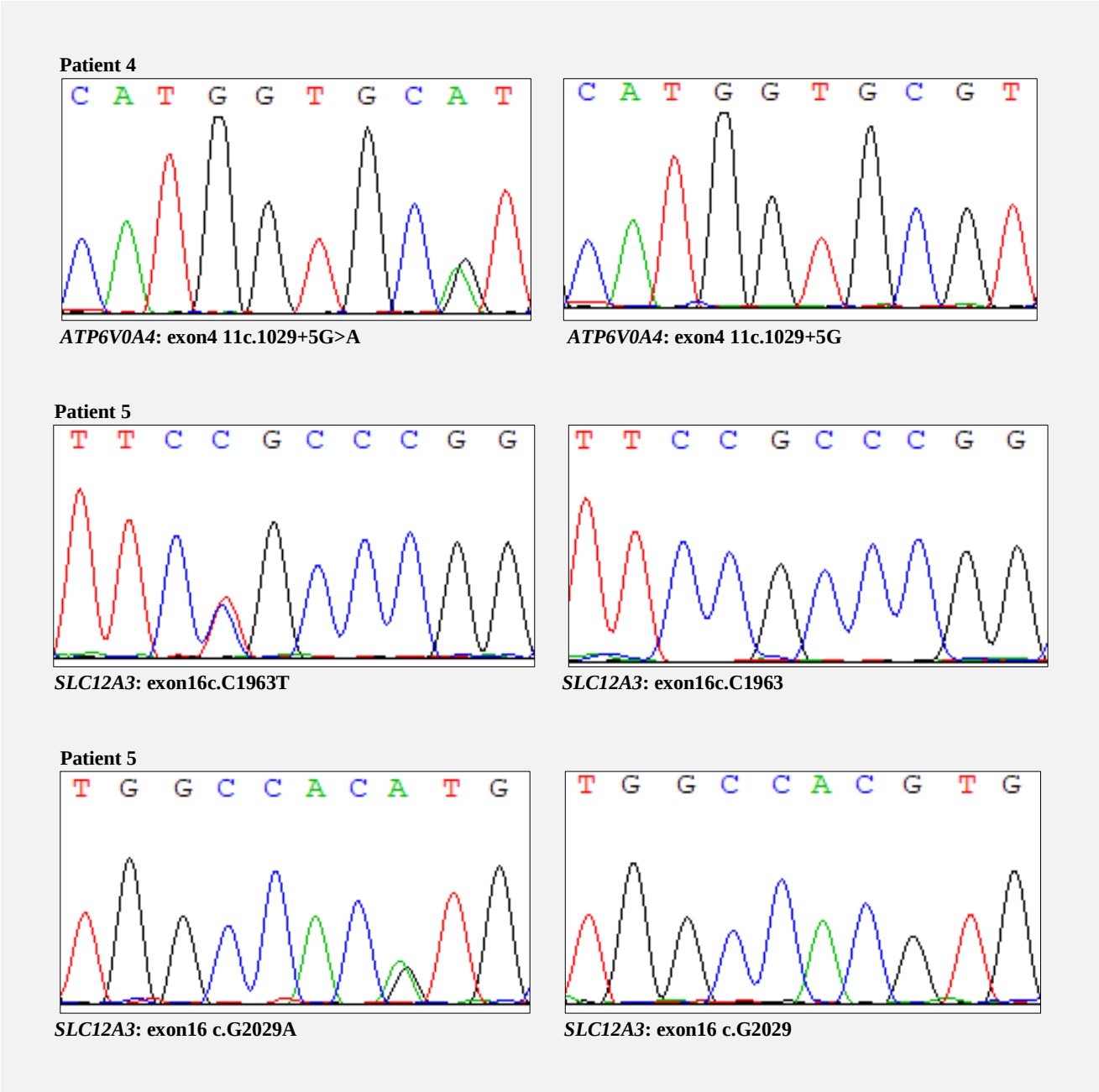


Figure 1. Sequence maps of five cases.

Mutant type in left column, wild type in right column.

ronolactone and Amphotericin, to improve treatment efficacy. Notably, the goal of BS treatment is not to normalize blood potassium but to achieve a level of ≥ 3.0 mmol/L, which is sufficient to alleviate the clinical symptoms of hypokalemia.

dRTA is characterized by impaired urinary acidification due to the inability of the distal renal tubules to excrete H^+ into the urine [8]. dRTA patients present with hyperchloremic metabolic acidosis, which is usually accompanied by a normal anion gap, hypokalemia, growth impairment, growth retardation, rickets, and renal calculi or renal calcium deposits [9]. Most cases of dRTA are caused by mutations in the *SLC4A1*, *ATP6V1B1*, and *ATP6V0A4* genes [10]. Some patients also present with sensorineural hearing loss, and studies have shown that the prevalence of sensorineural deafness among patients with *ATP6V0A4* mutations is 56.7% [11]. Patient 3 in this study harbored a rare heterozygous mutation

c.G298A in exon 4 of the *ATP6V1B1* gene responsible for renal tubular acidosis, which is mainly characterized by hypokalemia. Although sensorineural deafness was not detected in this patient, audiological assessment will be carried out regularly during follow-up.

Patient 4 in this study carried the heterozygous mutation c.G893A in exon 4 of the BS gene *BSND* and a heterozygous mutation c.1029+5G>A in exon 11 of the dRTA gene *ATP6V0A4*; these genes are involved in two renal tubular disorders of low morbidity, which is a valuable finding of two autosomal recessive genome synthesis composite gene disorders. The patient's clinical manifestations were lack of specificity, with low potassium levels, a normal pH, and a normal renin-angiotensin-aldosterone system; this condition is difficult to diagnose clearly by relying on conventional biochemical tests but can easily be genetically diagnosed.

The main aim of primary dRTA treatment is to correct metabolic acidosis and avoid complications, and alkaline substances with citrate and/or potassium bicarbonate should be administered. The patient's main clinical manifestations were recurrent malaise, intractable hypokalemia, and muscle weakness in the lower extremities; the patient had no significant hearing loss and no kidney stones. Hypokalemia was corrected with potassium supplementation, and the symptoms of malaise resolved. GS patients present with familial hypokalemia and hypomagnesemia, and the main clinical features are mainly hypokalemia, metabolic alkalosis, hypomagnesemia, and hypocalciuria [5]. Among them, hypocalcemia and hypomagnesemia are considered the main differences from BS, but this determination is not reliable. With the progress of molecular genetics, Gitelman, an American doctor, first reported the genes that are mutated in GS in 1966, which provided sufficient evidence to confirm the diagnosis of GS. One study further demonstrated the importance of *SLC12A3* gene screening for GS by exploring the phenotype and genotype of four family lines of GS patients [12].

GS is an inherited autosomal recessive tubulopathy involving salt loss caused by mutations in the *SLC12A3* gene on the long arm of chromosome 16, resulting in inactivating variants in the double allele of the *SLC12A3* gene encoding the thiazide-sensitive sodium chloride cotransporter protein, which is expressed only in the apical membrane of the cells lining the distal tubule. Studies have shown that 488 mutations in the *SLC12A3* gene have been identified in GS patients, including missense mutations, shear mutations, deletion mutations, nonsense mutations, reading frame shift mutations, and other mutations. Most of the mutations were compound heterozygous mutations, with missense mutations being the most common. Patient 5 harbored the compound heterozygous mutation of c.C1963T and c.G2029A in exon 16 of *SLC12A3*, which is causative of GS. Patient 5 had hypocalcemia and hypomagnesemia, which were consistent with the typical manifestations of GS. GS progresses slowly and has an overall good prognosis, but long-term progression still affects quality of life, and there is a risk of developing renal insufficiency. Therefore, early detection and timely treatment through genetic testing are highly important. Currently, GS is incurable, and clinical treatment of GS is based on symptomatic management through oral supplementation of potassium chloride and potassium magnesium methylate.

This study also provides important clues for further exploring the pathogenesis of clinically difficult hypokalamia. However, the results of this study may have several limitations due to the small sample size. Further in-depth studies with larger sample sizes are needed in the future to reveal more about the pathogenic mechanisms and clinical features of renal hypokalemia.

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Declaration of Interest:
The authors have no conflicts of interest to declare.

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