

Three Novel Homozygous Mutations of the *SLC12A3* Gene in a Gitelman Syndrome Patient

Mei Zhong^{1,*}
Zhenwei Zhai^{2,*}
Xing Zhou¹
Jingxia Sun¹
Hui Chen¹
Wensheng Lu¹

¹Department of Endocrinology, The Peoples Hospital of Guangxi Zhuang Autonomous Region, Guangxi, People's Republic of China; ²Department of Endocrinology, Tongde Hospital of Yuncheng, Changzhi Medical College, Shanxi, People's Republic of China

*These authors contributed equally to this work

Aim: Gitelman syndrome (GS) is an autosomal recessive disorder characterized by hypokalemic metabolic alkalosis. In this study, we investigated the clinical presentation and sequenced 26 exons of *SLC12A3* gene in a patient with a clinical suspicion of GS.

Methods: Clinical work-up including clinical examination, electrocardiography (ECG), chest X-ray, bone mineral density (BMD), and ultrasound examination was conducted and all exons of *SLC12A3* gene were analyzed by whole-exome sequencing.

Results: The patient showed hypokalemia, hypomagnesemia, and metabolic alkalosis and was found to have four novel homozygous missense mutations including one known mutation (c.1456 G>A in exon 12) and three novel mutations (c.366A > G in exon 2, c.791C > G in exon 6 and c.1027C > T in exon 8).

Conclusion: Four mutation sites of *SLC12A3* gene were found in the patient, three of which have not been reported before. These results may be useful for better understanding the function of this gene and can assist clinicians with treatment decision-making.

Keywords: Gitelman syndrome, clinical characteristics, *SLC12A3* gene, exome sequencing, gene mutation

Introduction

Gitelman syndrome (GS) is an autosomal recessive inherited disease initially reported in 1996 and is a rare renal tubular disorder with a prevalence of 1:40,000.^{1,2} GS is closely associated with the mutations of *SLC12A3* gene coding for the thiazide-sensitive sodium-chloride cotransporter (NCCT) of the distal convoluted tubule (DCT).³ To date, more than 400 mutations of *SLC12A3* have been identified in GS.⁴⁻⁸ Most of the mutations are missense, splice-site, nonsense, frameshift, deletions, and insertion mutations.⁹

The main clinical manifestations of GS are hypokalemic metabolic alkalosis, hypomagnesemia, hypocalciuria and renin-angiotensin-aldosterone system (RAAS) activation. Besides, patients with GS may present with spasticity, muscle weakness, paresthesia, numbness, polyuria, and palpitation.¹⁰ However, GS lacks specific clinical manifestations and is easily confused with other diseases such as Bartter syndrome, renal tubular acidosis, and primary aldosteronism.^{11,12} In addition to history taking and clinical work-up, genetic testing is an important and effective tool for diagnosing GS in clinical practice.¹³ Here, our present study aimed to analyze the clinical features and genetic characteristics of a Chinese female patient with GS.

Correspondence: Wensheng Lu
Tel +86 17634045555
Email lwsxqz@163.com



Methods

Subject and Clinical Characteristics

A 60-year-old female patient was admitted to the Department of Endocrinology in Tongde Hospital of Shanxi province because of recurrent hypokalemia. She had no obvious clinical symptoms such as palpitations, shortness of breath, muscle weakness of the lower limbs, vomiting, nausea, anorexia, diarrhea, polyphagia and emaciation and denied a history of chronic kidney disease. She was treated with potassium citrate or potassium chloride, but she had no history of other drugs such as diuretics, proton pump inhibitors, and anti-tumor agents.

Clinical Examinations

The patient underwent detailed systemic physical examinations. Fasting blood samples and urine samples were obtained. The electrolytes of the blood and urine, plasma angiotensin, plasma renin activity, and plasma aldosterone were analyzed at the central chemistry laboratory of Tongde Hospital of Shanxi province. Besides, ECG, chest X-ray, bone mineral density (BMD) examination and ultrasound examination were conducted.

Genetic Analysis

The diagnosis of Gitelman syndrome was based on the clinical symptoms, biochemical measurements and analysis of genetic mutations of *SLC12A3* gene. Genomic DNA was extracted from peripheral blood samples of the female patient using a nucleic acid extraction kit (NO. BST01051, BaiO Technology Co., Ltd, Shanghai, China) according to the manufacturer's protocol. The *SLC12A3* gene was screened for mutations using Sanger sequencing. The nucleotide sequences of the PCR products were aligned to the UCSC database using SnapGee software (v3.2.1)

Results

Baseline Clinical Characteristics of the Subject

Blood pressure, heart rate, and body mass index (BMI) of the patient were 128/70 mmHg, 76 beats per minute, and 26.7 kg/m², respectively. Systemic physical examinations showed no abnormalities. The results of laboratory tests of the patient are listed in Table 1. Laboratory assays revealed hypokalemia, hypomagnesemia, hypercalcemia, and metabolic alkalosis. Furthermore, the patient had elevated parathyroid hormone (PTH) and plasma renin activity and angiotensin II. 24-h urine analysis showed that the

Table 1 Laboratory Tests of the Subject

Variable	Test Value	Reference Range
Blood tests		
Potassium (mmol/L)	2.91*	3.5–5.5
Sodium (mmol/L)	143	137–147
Chloride (mmol/L)	101	99–110
Calcium (mmol/L)	2.72*	2.15–2.55
Magnesium (mmol/L)	0.49*	0.66–0.99
Phosphate (mmol/L)	1.04	0.85–1.51
PTH (pg/mL)	86.5*	15–65
TPINP (ng/mL)	48.48	20.25–76.31
β-CTX (ng/mL)	0.26	0.131–0.9
25(OH)D (ng/mL)	29.24	>20
Arterial blood gas analysis		
PH	7.52*	7.35–7.45
PO ₂ (mmHg)	61*	80–100
PCO ₂ (mmHg)	34*	35–45
Bicarbonate (mmol/L)	27.4*	18–23
Renin–angiotensin–aldosterone system		
Angiotensin II (pg/mL)	56.94*	28.2–52.2
Plasma renin activity (ng/mL/h) (supine)	5.48*	0.05–0.79
Plasma aldosterone (ng/mL) (supine)	0.16	0.059–0.174
Plasma renin activity (ng/mL/h) (upright)	1.08	0.93–6.56
Plasma aldosterone (ng/mL) (upright)	0.17	0.065–0.296
24-h urine tests		
Potassium (mmol/24 h)	89.4	25–125
Calcium (mmol/24 h)	3.31	2.5–7.5
Sodium (mmol/L)	188.65	130–260
Phosphate (mmol)	17.49	22–48

Note: *Abnormal value.

Abbreviations: PTH, parathyroid hormone; TPINP, total N-terminal propeptide of type I procollagen; β-CTX, β-C-terminal telopeptides of type I collagen; 25(OH) D, 25-hydroxyvitamin D.

levels of urinary potassium and urinary calcium were normal. ECG revealed normal sinus rhythm, ST segment and T-waves abnormality but had no prolongation of the QT interval. The BMD data showed there was a significant decrease at the left forearm. Chest X-ray and ultrasound (thyroid, carotid, heart, abdomen, and pelvic cavity) did not show any obvious abnormalities (data not shown).

Genetic Analysis

Sanger sequencing of *SLC12A3* gene was performed in the subject. Genetic analysis showed 4 mutations in the exons of *SLC12A3* gene: c.366A > G in exon 2, c.791C > G in exon 6, c.1027C > T in exon 8, and c1456G>A in exon 12 (Figure 1).

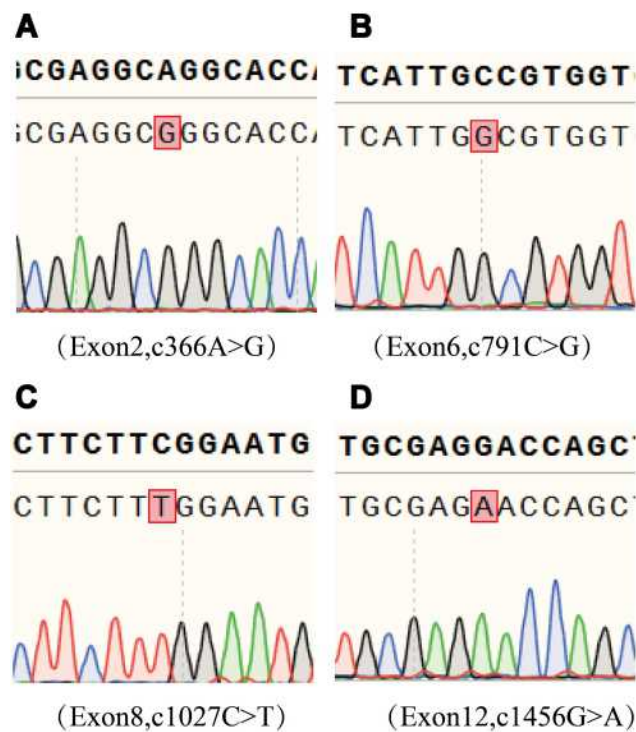


Figure 1 Genetic analysis of the *SLC12A3* gene. (A) c.366A > G in Exon 2. (B) c.791C > G in Exon 6. (C) c.1027C > T in Exon 8 and (D) c.1456G > A in Exon 12. The mutant nucleotides are marked in the red frames.

Clinical Treatment of the Subject

After the diagnosis, spironolactone and potassium citrate were used to treat hypokalemia and potassium magnesium aspartate was used to treat hypomagnesaemia. The patient was discharged with normokalemia and no other discomfort following one week of treatment.

Discussion

In the present study, we reported the case of a 60-year-old Chinese female patient with GS. The biochemical examination showed hypokalemia, metabolic alkalosis, hypomagnesemia, hypercalcemia, hyperreninemia, elevated angiotensin II, and PTH levels. ECG showed normal sinus rhythm, ST segment and T-waves abnormality but had no prolongation of the QT interval. Besides, BMD was decreased in the left forearm. Genetic analysis identified four mutations of *SLC12A3* gene, c.366A > G in exon 2, c.791C > G in exon 6, c.1027C > T in exon 7, and c1456G>A in exon 12. The treatment with supplements of potassium and magnesium improved hypokalemia and hypomagnesemia.

GS is a rare inherited salt-wasting disorder characterized by hypokalemic metabolic alkalosis with hypomagnesemia, hypocalciuria and RAAS activation. GS is caused by mutations in *SLC12A3* gene coding for the thiazide-sensitive

NCCT of the DCT, which leads to a decrease in sodium reabsorption, increases potassium and hydrogen excretion and therefore develop hypokalemic metabolic alkalosis.¹⁴ The enhanced passive Ca^{2+} transport in the proximal tubule leads to hypocalciuria¹⁵ and the downregulation of the epithelial Mg^{2+} channel transient receptor potential channel subfamily M, member 6 (Trpm6) is a possible mechanism involved in hypomagnesemia.¹⁶ The mechanism of hypocalciuria is uncertain, but some studies have found that one reason for it may be hypovolemia. Meanwhile, hypovolemia activates RAAS.^{17,18} The patient had hypokalemia, metabolic alkalosis, hypomagnesemia, hyperreninemia, and elevated angiotensin II level, which was consistent with the clinical manifestations of GS. In the study, the elderly female had decreased BMD. Postmenopausal women are prone to develop hypocalcemia and postmenopausal osteoporosis, which is caused by estrogen deficiency after menopause. Long-term hypocalcemia may overstimulate the parathyroid gland and lead to secondary hyperparathyroidism.

Genetic identification is the golden standard for the diagnosis of GS. We identified compound mutations of *SLC12A3*, c.366A > G in exon 2, c.791C > G in exon 6, c.1027C > T in exon 7, and c1456G>A in exon 12. The c1456G>A in exon 12 is reported as a hotspot mutation of *SLC12A3*. A heterozygous mutation, c.366A > G in the gene has been reported. But c.366A > G in exon 2 was a homozygous mutation in this study. Moreover, c.791C > G and c.1027C > T are two novel mutations. Hence, we performed a complementary study on the mutations of *SLC12A3*.

GS and Bartter syndrome (BS) show extremely similar clinical and laboratory manifestations including hypokalemia, metabolic alkalosis, hyperreninemia, and hyperaldosteronemia. But BS presents with an early age of onset and exhibits apparent clinical symptoms.¹² Simultaneously, BS is closely related to the mutations of *CLCNKB* gene. They can be differentiated by clinical manifestation and genetic tests.^{19,20}

Our present study has several limitations. First, the mutation was detected in only one patient but not in pedigree. Further research should be performed in the pedigree. Second, a further exploration is needed to find the correlation between genotype and phenotype and then provide better understanding of GS. Moreover, more experiments are needed to reveal the underlying molecular mechanism of GS.

Conclusions

Overall, our study identified four mutations of *SLC12A3* gene in a Chinese female patient and three of the mutations were novel. These findings might be useful for better

understanding the function of this gene and aid with diagnosis and treatment decisions.

Ethics Approval and Consent to Participate

The study was conducted in compliance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of Tongde Hospital of Shanxi province. The patient provided informed consent for the case details to be published.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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