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# Three uncommon mutations of the SLC12A3 gene in gitelman syndrome: case reports and review of the literature

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## Abstract

**Background:** Gitelman syndrome is a rare autosomal recessive salt-wasting tubulopathy characterized by low potassium and magnesium levels in the blood, decreased excretion of calcium in the urine, and metabolic alkalosis. It is commonly caused by an inactivating mutation in the SLC12A3 gene (16q13), which encodes a thiazide-sensitive sodium chloride cotransporter. Here, we present three cases with the same clinical and laboratory findings that showed different mutations in the SLC12A3 gene.

**Case presentation:** Three children, a 14-year-old boy, a 7-year-old girl, and an 11-year-old boy, were admitted to our hospital at different times with nausea, weakness, muscle cramps in hands, and failure to thrive complaints. Blood tests showed hypokalemia, hypomagnesemia and metabolic alkalosis. Patients were referred to Pediatric Nephrology Clinic and diagnosed with Gitelman syndrome. Genetic tests of three cases showed homozygous mutations of c.1928C > T, p.Pro643Leu, c.248G > A, p.Arg83Gln, and c.1919A > G, p.N640S in the SLC12A3 gene exists, respectively. Potassium chloride, magnesium replacements, and indomethacin were given for treatment to patients. During follow-up, patients' heights and weights were increased dramatically, and nausea complaints were over.

**Conclusion:** Different mutations in the SLC12A3 gene in Gitelman syndrome can be detected but clinical, and laboratory findings were generally similar. Treatment with potassium, magnesium supplements, and indomethacin showed significant improvements in symptoms.

**Keywords:** Gitelman syndrome, Hypokalemia, Hypomagnesemia, SLC12A3 gene mutations

## Background

Gitelman syndrome (GS) is an inherited autosomal recessive salt-wasting tubulopathy characterized by low potassium and magnesium levels in the blood, decreased excretion of calcium in the urine, and elevated blood pH [1, 2]. Complaints and findings in GS usually appear in late childhood or adolescence. The most common complaints are excruciating muscle spasms (tetany), muscle weakness or cramps, dizziness, and salt craving [3]. It is commonly caused by an inactivating mutation in the SLC12A3 gene (16q13), which encodes a thiazide-sensitive sodium-chloride cotransporter (NCCT) [4]. These channels are located in the distal convoluted tubule of the kidney.

In the present study, we retrospectively report three cases of GS with uncommon different homozygous mutations in the SLC12A3 gene. Demographic findings of patients were variable, but symptoms and laboratory findings were typical. Another aim of this study is to report three cases of GS with a literature review.

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## Case presentation

**Case 1**: A 14-year-old boy was admitted to the hospital due to nausea and failure to thrive complaints. Demographic and laboratory findings of the case are shown in Table 1. Weight was 40 kg (3rd–10th percentile), and height was 137 cm (below 3rd percentile). Blood pressure was 110/72 mmHg (< 90th percentile), and heart rate was 82 beats/min. Blood tests showed hypokalemia (2.7 mmol/L), hypomagnesemia (1.33 mg/dL). Other laboratory findings of the patient were within normal limits; calcium 10.3 mg/dL, sodium 136.5 mmol/L, chloride 98.7 mmol/L, urea 19 mg/dL, creatinine 0.76 mg/dL and urine calcium/creatinine ratio was 0.08 mg/mg. Blood samples for renin and aldosterone were drawn in the supine position, and the results were higher than the normal range. Blood gas showed metabolic alkalosis; pH was 7.51, and bicarbonate ($HCO_3$) was 32 mmol/L. Imaging studies and electrocardiography were unremarkable. Urinary tract ultrasonography, audiometric and ophthalmologic assessment was normal. According to the clinical and biochemical results, a diagnosis of GS was suspected. Written informed consent has been given to the parents prior to genetic testing. We performed next-generation sequencing analysis and Sanger sequencing to explore the SLC12A3 mutations in the patient. Genetic tests showed c.1928 C > T (pPro643Leu), a novel SLC12A3 gene homozygous mutation in DNA sequence analysis. The patient was treated with oral potassium (5 mEq/L/day), magnesium (10 mg/kg/day) and indomethacin (2 mg/kg/day). In the follow-up 1 month after the treatment, his serum potassium and magnesium became within the.

reference range, and 6 months later, the patient exhibited a 10 kg weight gain and a 12 cm height gain (Table 1).

**Case 2**: A 7-year-old girl was admitted to the hospital with weakness, spasms in the hands and legs, and failure to thrive complaints. The demographic and laboratory findings of the patient are shown in Table 1. On physical examination, body weight was 18 kg (3rd–10th percentile), height was 112 cm (3–10th percentile), blood pressure was 100/60 mmHg (< 90th percentile), heart rate was 78 beats/min, and other physical examination findings were normal. Blood tests showed hypokalemia (2.8 mmol/L [normal range 3.5–5.2 mmol/L]), hypomagnesemia (1.19 mg/dL [normal range: 1.7–2.2 mg/dL]) and hypochloremia (88 mmol/L [normal range 98–107 mmol/dL]). Other laboratory findings of the patient were within normal limits; creatinine: 0.51 mg/dL, sodium: 138 mmol/L, calcium: 10.1 mg/dL, urine calcium/creatinine ratio: 0.09 mg/mg. Blood gas showed metabolic alkalosis; pH: 7.56 and $HCO_3$: 33 mmol/L. Blood samples for renin and aldosterone were drawn in the supine position, and the results were higher than the normal range. Imaging studies and electrocardiography were unremarkable. Urinary tract ultrasonography, audiometric and ophthalmic examinations were normal. Written informed consent has been given to the parents prior to genetic testing. We performed next-generation sequencing analysis and Sanger sequencing to explore the SLC12A3 mutations in the patient.

**Table 1** Demographic data and laboratory findings of three cases

| Parameters | Reference values | Case 1 | | Case 2 | | Case 3 | |
|---|---|---|---|---|---|---|---|
| | | At admission | Third month | At admission | Third month | At admission | Third month |
| Weight (kg) | – | 40 | 50 | 18 | 23.6 | 27 | 31 |
| Height (cm) | – | 137 | 149 | 112 | 118 | 128 | 135.9 |
| pH | 7.35–7.45 | 7.51* | 7.46* | 7.56* | 7.44 | 7.49* | 7.39 |
| $HCO_3$ (mmol/L) | 22–26 | 32* | 27.8 | 33* | 27.8 | 33.6* | 25.8 |
| Na (mmol/L) | 135–145 | 136.5 | 141.5 | 138 | 141.5 | 138.5 | 142.5 |
| K (mmol/L) | 3.5–5.5 | 2.7* | 4.0 | 2.8* | 3.8 | 2.94* | 3.92 |
| Cl (mmol/L) | 97–107 | 98.7* | 102.4 | 88.0* | 101.7 | 86* | 99.4 |
| Calcium (mg/dL) | 8.5–10.3 | 10.3 | 10.3 | 10.1 | 10.7 | 10.4 | 10.8 |
| Mg (mg/dL) | 1.7–2.3 | 1.33* | 1.94 | 1.19* | 1.89* | 1.01* | 2.1* |
| uCa/uCr (mg/mg) | < 0.21 | 0.05* | 0.10 | 0.04* | 0.16 | 0.06* | 0.19 |
| Renin (pg/mL) | 2.5–45.1 | 62.08 | – | 51.02 | – | 66.01 | – |
| Aldosterone (pg/mL) | 20–230 | 254.03 | – | 263.04 | – | 248.02 | – |
| SLC12A3 gene Mutation | | c.1928 C > T, p.Pro643Leu homozygous mutation | | c.248G > A, p.Arg83Gln homozygous mutation | | c.1919A > G, p.N640S homozygous mutation | |

Na, Sodium; K, Potassium; Cl, Chloride; Mg, Magnesium; uCa, urine calcium; uCr, urine creatinine

*Abnormal values

Genetic tests showed (c.248G > A pArg83Gln homozygous mutation) in the SLC12A3 gene. The patient was diagnosed with GS. She was treated with oral potassium (5 mEq/L/day), magnesium (10 mg/kg/day) and indomethacin (2 mg/kg/day). In the follow-up, 1 month after the treatment, her serum potassium and magnesium became within the reference range, and 6 months later, she exhibited a 5.6 kg weight gain and a 6 cm height gain (Table 1).

**Case 3**: A 11-year-old boy was admitted to our clinic with weakness, lethargy, carpopedal spasm, and growth retardation complaints. The patient had no history of vomiting, diarrhea complaints, and diuretic or laxative use. The demographic and laboratory findings of the patient are shown in Table 1. On physical examination, his height was 128 cm (below the 3rd percentile), and her weight was 27 kg (3rd–10th percentile). Blood pressure was 110/74 mmHg (< 90th percentile). Blood gas showed metabolic alkalosis (pH: 7.49, $HCO_3$: 32 mmol/L) and laboratory tests showed hypokalemia (2.94 mmol/L [normal range 3.5–5.2 mmol/L]), hypomagnesemia (1.01 mg/dL [normal range: 1.7–2.2 mg/dL]) and hypochloremia (86 mmol/L [normal range 98–107 mmol/dL]). Serum creatinine level was normal (0.63 mg/dL). Blood samples for renin and aldosterone were drawn in the supine position, and the results were higher than the normal range. Renal ultrasonography was normal. Written informed consent has been given to the parents prior to genetic testing. We performed next-generation sequencing analysis and Sanger sequencing to explore the SLC12A3 mutations in the patient. Genetic tests showed a homozygous mutation in the SLC12A3 gene in DNA sequence analysis (c.1919A > G, p.N640S). The patient was diagnosed with GS. He was treated with oral potassium (5 mEq/L/day), magnesium (10 mg/kg/day) and indomethacin (2 mg/kg/day). In the follow-up, 1 month after the treatment, his serum potassium and magnesium became within the reference range, and 6 months later, he exhibited a 4 kg weight gain and a 7.9 cm height gain (Table 1).

## Discussion

Gitelman syndrome is also known as familial hypokalemic hypomagnesaemia because hypokalemia is the most common phenomenon. Gitelman syndrome is estimated to have a prevalence of 1 in 40,000 homozygous people, and males and females are affected equally [1, 5]. Symptoms usually appear after six but may not be present until adolescence or adulthood. Usually, the diagnosis is made incidentally by detecting hypokalemia in a routine blood sample. Since hypokalemia can cause cardiac arrest, respiratory muscle paralysis, and death, early diagnosis of GS is essential [1]. Diagnosis can be made fortuitously by detecting hypokalemia and hypomagnesemia during growth retardation identification. Patients may complain of fatigue, weakness, dizziness, thirst, muscle weakness or cramps, palpitations, and nocturia [3, 5].

Mutations in the SLC12A3 gene usually cause Gitelman syndrome. Less frequently, the condition is caused by mutations in the CLCNKB gene. More than 140 mutations in the SLC12A3 gene have been found in patients with GS [4, 6–10]. Three novel mutations, c.2 T > C, c.1609C > T and c.3055G > A, were identified by Wang et al. [9]. Proteins produced from these genes are involved in the renal reabsorption of salt (NaCl) from the urine back into the bloodstream. Mutations in both genes impair the renal ability to reabsorb salt, resulting in an excess salt loss in the urine. Abnormalities in salt transport also affect the reabsorption of other ions, including potassium, magnesium, and calcium ions. The electrolyte imbalance underlies the basic features of GS [1]. The SLC12A3 gene provides instructions for making a protein known as NCCT, which moves charged sodium and chloride atoms across cell membranes. NCCT is essential for normal renal function. Salt retention affects the body's fluid balance and helps maintain blood pressure. It is reported in the literature that 1% of the population is the carrier of the heterozygous SLC12A3 gene mutation [1]. For a parent with GS, the risk of passing the abnormal gene to their offspring is low, 1 in 400, unless both parents are carriers of the disease [6].

In our first case, a c.1928C > T (p.Pro643Leu) homozygous mutation was detected in the SLC12A3 gene. In the literature, the c.1928C > T (p.Pro643Leu) missense variant has been reported rarely in patients with GS. Control data are unavailable for this variant, which is reported at a frequency of 0.001774 in the Ashkenazi Jewish population of the Genome Aggregation Database. Based on the collective evidence, the p.Pro643Leu variant is classified as a variant of uncertain significance in GS [11–14]. This mutation would be expected to modify the protein structure and implicated in the loss of function of the NCCT of the distal tubule.

In our second case, a c.248G > A (p.Arg83Gln) homozygous mutation was detected in the SLC12A3 gene. This mutation is also not common among the SLC12A3 gene mutations. In a study, a c.248G > A (p.Arg83Gln) homozygous mutation in SLC12A3 has been reported in a 48-year-old male patient and his family [15]. In this patient, weakness in the knee and Achilles tendons, carpopedal spasm, arthralgia, hypokalemic alkalosis, mild renal dysfunction, hypomagnesemia, hypocalciuria, hyperuricemia, normotension, hyperreninemia, and chondrocalcinosis were detected.

In our third case, a c.1919A > G, p.N640S homozygous mutation was detected in the SLC12A3 gene. Although the gene mutation identified in this case has

been reported in the literature, its relation to GS has not been identified. Numerous gene mutations are reported in a large series study [4]. In this study, 448 index cases with the clinical diagnosis of GS were retrospectively screened for mutations in the SLC12A3 gene. Two affected alleles were detected in 315 (70%) of 448. However, 79 (25%) patients had homozygous mutations, and 236 (74.9%) patients had compound heterozygous mutations. In addition, only one mutant allele was detected in 81 (18%) patients and wild-type genotype in 52 (11.6%) patients.

There is no cure for GS. Treatment of GS includes supplements of potassium and magnesium. A high salt diet containing potassium and magnesium supplements to normalize blood levels is the basis of treatment [1]. Severe potassium and magnesium deficiencies may require intravenous replacement. If low blood potassium levels cannot be raised sufficiently with oral supplements, aldosterone antagonists (spironolactone or eplerenone) or epithelial sodium channel blockers such as amiloride could reduce the urinary excretion of potassium [1, 2]. Indomethacin may also be used in patients with early onset of the disease, such as infants and children [16]. Cardiac evaluation should be done to prevent dysrhythmias and to monitor QT intervals. In these patients, drugs that prolong the QT interval (macrolides, antihistamines, beta-2 agonists) should be avoided [17].

## Conclusions

In this report, we presented three cases with three different mutations of the SLC12A3 gene. GS should be considered in older children with hypokalemia, hypochloremia, hypomagnesemia, and metabolic alkalosis. The most common complaint was growth retardation in our cases. GS needs to be considered in patients admitted to Pediatric outpatient clinics with failure to thrive complaints. Patients with GS may present different clinical pictures depending on the gene mutation, but their laboratory findings are generally similar. Further investigations are needed to understand the underlying genetic basis.

#### Abbreviations

GS: Gitelman syndrome; NCCT: Sodium-chlorine cotransporter; $HCO_3$: Bicarbonate.

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#### Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by MAG, FED and HD. The first draft of the manuscript was written by MAG and FED, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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#### Availability of data and materials

All data used during this report are included in this published article. Further data are available from the corresponding author on reasonable request.

## Declarations

#### Ethics approval and consent for publication

This is a retrospective study. In addition, it is a case series and literature review. Therefore, we did not receive ethical approval. We only obtained written permission from the child and his family to publish it.

#### Consent to participate

Written consent was taken from the patient of the index case for publication.

#### Competing interests

The authors declare no competing interest.

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#### References

1. Blanchard A, Bockenhauer D, Bolignano D, Calò LA, Cosyns E, Devuyst O et al (2017) Gitelman syndrome: consensus and guidance from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int 91(1):24–33
2. Graziani G, Fedeli C, Moroni L, Cosmai L, Badalamenti S, Ponticelli C (2010) Gitelman syndrome: pathophysiological and clinical aspects. QJM 103(10):741–748
3. Gitelman HJ, Graham JB, Welt LG (1969) A familial disorder characterized by hypokalemia and hypomagnesemia. Ann N Y Acad Sci 162(2):856–864
4. Vargas-Poussou R, Dahan K, Kahila D, Venisse A, Riveira-Munoz E, Debaix H et al (2011) Spectrum of mutations in Gitelman syndrome. J Am Soc Nephrol 22(4):693–703
5. Knoers NV, Levtchenko EN (2008) Gitelman syndrome. Orphanet J Rare Dis 3:22
6. Hsu YJ, Hoenderop JG, Bindels RJ (2007) TRP channels in kidney disease. Biochim Biophys Acta 1772(8):928–936
7. Knoers NV (2009) Inherited forms of renal hypomagnesemia: an update. Pediatr Nephrol 24(4):697–705
8. Jiang L, Peng X, Zhao B, Zhang L, Xu L, Li X et al (2020) Frequent SLC12A3 mutations in Chinese Gitelman syndrome patients: structure and function disorder. Endocr Connect 11(1):e210262
9. Wang F, Guo M, Li J, Ma S (2021) Novel mutations of the SLC12A3 gene in patients with Gitelman syndrome. Scand J Clin Lab Invest 81(8):629–633
10. Zheng X, Shang S, Cai G, Chen X, Li Q (2021) Genetic analysis of SLC12A3 gene and diagnostic process in patients with Gitelman syndrome. Clin Nephrol 96(3):165–174
11. Nakhoul F, Nakhoul N, Dorman E, Berger L, Skorecki K, Magen D (2012) Gitelman's syndrome: a pathophysiological and clinical update. Endocrine 41(1):53–57
12. Cruz D, Shaer A, Bia M, Lifton R, Simon D, Yale Gitelman's and Bartter's Syndrome Collaborative Study Group (2001) Gitelman's syndrome revisited: an evaluation of symptoms and health-related quality of life. Kidney Int 59(2):710–717

13. Cruz DN, Simon DB, Nelson-Williams C, Farhi A, Finberg K, Burleson L et al (2001) Mutations in the Na-Cl cotransporter reduce blood pressure in humans. Hypertension 37(6):1458–1464
14. Yu RZ, Chen MS (2020) Gitelman syndrome caused by a rare homozygous mutation in the SLC12A3 gene: a case report. World J Clin Cases 8(18):4252–4258
15. Mou L, Wu F (2021) Simultaneous homozygous mutations in *SLC12A3* and *CLCNKB* in an inbred Chinese pedigree. Genes (Basel) 12(3):369
16. Liaw LC, Banerjee K, Coulthard MG (1999) Dose related growth response to indometacin in Gitelman syndrome. Arch Dis Child 81(6):508–510
17. Cortesi C, Lava SA, Bettinelli A, Tammaro F, Giannini O, Caiata-Zufferey M et al (2010) Cardiac arrhythmias and rhabdomyolysis in Bartter-Gitelman patients. Pediatr Nephrol 25(10):2005–2008

## Publisher's Note