Identification of novel compound mutations of SLC12A3 gene in a Chinese pedigree with Gitelman's syndrome exhibiting Bartter's syndrome-like phenotypes

CURRENT STATUS: UNDER REVIEW

BMC Nephrology

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DOI: 10.21203/rs.3.rs-20998/v1

SUBJECT AREAS
Urology & Nephrology

KEYWORDS
Hypokalemia; Gitelman's syndrome; SLC12A3; Hypercalciuria
Abstract

Background

Gitelman's syndrome (GS) is a rare salt-losing renal tubular disorder associated with SLC12A3 gene mutations, which encodes the Na-Cl co-transporter (NCCT). GS is characterized by hypokalaemic metabolic alkalosis, hypomagnesemia, hypocaliuria and elevated renin-angiotensin-aldosterone (RAA) level. The variability of phenotypes is likely to be associated with the variety of SLC12A3 mutations.

Methods

In this study, we reported the clinical features and the genetic analysis of a GS family pedigree.

Results

We identified novel mutations of SLC12A3, with c.433 C>T (p.Arg145Cys), c.1077 C>G (p.Asn359Lys), and c.1666 C>T (p.Pro556Ser). The proband exhibited hypokalaemia, hypomagnesemia, metabolic alkalosis, but hypercalcuria and kidney stone. The increased urinary calcium excretion made it confused to Bartter's syndrome (BS). The persistent renal potassium wasting associated renal tubular lesions finally affected urinary calcium reabsorption, leading to the increased calcium excretion. Genetic analysis revealed mutations of SLC12A3 with C433T (Arg145Cys, Het), C1077G (Asn359Lys, Het), and C1666T (Pro556Ser, Het). Those missense mutations led to the predicted amino acid change, caused differences of NCCT protein structures and function. One sister of the proband carried the same mutant sites, however, exhibited milder phenotypes including hypokalemia, hypomagnesemia, RAAS activation, but not elevated urinary calcium excretion. With administration of antisterone, potassium chloride and magnesium supplement, the serum potassium and magnesium were maintained in normal ranges.

Conclusions

In this study, we identified the novel mutations of SLC12A3 and the varieties of clinical features. Further efforts are needed to investigate the diversity in clinical manifestations of GS and its correlation with SLC12A3 mutations.

Background
Gitelman's syndrome (GS, OMIM#263800) is an autosomal recessive inherited, salt-losing renal tubular disorder, with the clinical features including hypokalemia, renal potassium loss, metabolic alkalosis, hypomagnesemia, hypocalciuria with normal blood pressure [1]. GS is associated with mutations of SLC12A3 (solute carrier family 12 member 3) gene, which locates in chromosome 16q13 and encodes the thiazide-sensitive Na-Cl cotransporter (NCCT) of distal convoluted tubule [2]. Till now, there are more than 400 varieties of SLC12A3 related to GS have been reported [2, 3, 4, 5]. Among those mutations include nonsense, missense, deletion, insertion, and splice-site, missense mutations account for the most in GS [6]. Most SLC12A3 mutations in GS are simple or complex heterozygous mutations and few of them are homozygous [7].

The clinical symptoms of GS are variable, including muscle weakness, paresthesias, numbness, polyuria, and growth retardation in children [2]. Some patients are asymptomatic or mildly symptomatic, or only exhibit non-specific fatigue, leading to the miss diagnosis or misdiagnosis. Severe and persistent hypokalemia may lead to glucose intolerance, cardiac and renal dysfunction. Disordered renal reabsorption of sodium and chloride leads to a series of pathophysiological changes and clinical manifestations, including decreased blood volume, and activated renin-angiotensin aldosterone system (RAAS). GS and Bartter's syndrome (BS) both exhibit hypokalemia, metabolic alkalosis, and normal blood pressure with high plasma renin activity and high aldosterone concentration. They exhibit similar phenotypes, which make it difficult to diagnose. Urinary calcium excretion is considered to be an important clue to distinguish these two diseases [8].

In this study, we identified novel compound hybrid mutations of SLC12A3 with c.433 C>T (p.Arg145Cys), c.1077 C>G (p.Asn359Lys), and c.1666 C>T (p.Pro556Ser) in a family pedigree of GS. The proband presented hypercalcuria and renal calcification. The further genotype-phenotype correlation analysis is needed to provide deeper insights into GS.

Methods

Patients’ recruitment

Seven participants (three men and four women) from a Chinese family were recruited. The diagnosis of GS depends on clinical symptoms, biochemical parameters and genetic analysis. All participants
denied a history of laxatives, diuretics, or other drugs including insulin, β-receptor activator or Chinese medicinal herbs. This study was approved by the ethics committee of the Affiliated Hospital of Qingdao University.

Genetic analysis
Genomic DNA was extracted from peripheral blood sample using the QIAamp Blood DNA Mini Kit (QIAGEN, USA) according to the manufacturer’s protocol. After amplification using 2X polymerase chain reactions (PCR) MasterMix polymerase (Tiangen, China) by ABI9700 PCR (Life technology, USA), the products were captured and purified with Panel probe (illumine Inc. USA), then directly sequenced on the ABI 3500 automated DNA sequencer (Life technology, USA). Mutations of SLC12A3 were detected using next generation sequencing (NGS) and subsequently confirmed using Sanger sequencing.

Results
Clinical presentation
A 42-year-old male was presented to our hospital with fatigue, repeated muscle weakness and quadriplegia for ten years. Laboratory investigation exhibited hypokalemia, hypomagnesemia, increased urinary potassium excretion, activated renal-aldosterone system, hypercalcaemia and hypercalcuria (Table 1). The fraction excretion rate of potassium (FEK%) was significantly increased to 30.5–49.2% (normal range 8–12%), suggesting that hypokalemia resulted from renal potassium wasting. Thyroid function, cortical and adreno-corticotropic hormone (ACTH) were normal. Other possible causes of hypokalaemia such as thyrotoxic periodic paralysis, renal tubule acidosis, Cushing’s syndrome were excluded. Serum calcium slightly increased, and urinary calcium excretion was also increased (FECa 2.66%, urinary calcium to creatine ratio 0.70) (Table 1), and intact parathyroid hormone (PTH) was suppressed. The glucose stimulated insulin secretion (GSIS) test suggested the delayed insulin release and insulin resistance. Renal calcification was detected by computed tomography (CT) (Fig. 1A-D). Kidney biopsy revealed that individual renal tubules showed large vacuolar degeneration or atrophy (Fig. 1E-H), suggesting that renal tubule lesions were associated with persistent renal potassium wasting.
Table 1
Biochemical profiles of the proband and his sister diagnosed with GS.

|                  | II-4  | II-6 (proband) | Normal range |
|------------------|-------|----------------|--------------|
| **Age/Sex**      | 48F   | 42M            |              |
| **Biochemistry** |       |                |              |
| Serum K (mmol/L) | 3.08↓ | 1.9–2.8↓↓      | 3.5–5.5      |
| Serum Mg (mmol/L)| 0.68↓ | 0.71↓          | 0.8–1.02     |
| Serum Ca (mmol/L)| 2.5   | 2.48–2.6       | 2.1–2.52     |
| FE K (%)         | 12.66 | 30.5–49.2↑↑    | 8–12%        |
| uCa/Cr           | 0.03–0.07↓↓ | 0.71↑  | < 0.2        |
| FE Ca (%)        | 0.05–0.14↓↓ | 2.66↑    | < 1%         |
| PTH (pg/mL)      | 32.57 | 5.69          | 15–65        |
| Fasting blood glucose (mmol/L) | 5.60 | 7.42        |
| OGGT-2 h BG (mmol/L) | -   | 11.2        |
| Fasting C-peptide (ng/mL) | -   | 4.07        |
| OGGT-2 h C-peptide (peak) | -   | 15.41        |
| RAAS (lying condition) |       |              |              |
| Renin (ng/mL/hr) | 2.47  | 3.58          | 0.15–2.33    |
| Aldosterone (pg/mL) | 79.53 | 123.72       | 30–160       |
| RAAS (standing condition) |       |              |              |
| Renin            | 9.36  | > 13.56       | 0.10–6.56    |
| Aldosterone      | 143.5 | 279.65        | 70–300       |
| Arterial blood gas |       |              |              |
| pH               | 7.38  | 7.4           | 7.35–7.45    |
| Bicarbonate (mmol/L) | 24   | 26           | 22–28        |

The patient was supplied with potassium chloride sustained release tables, antisterone and magnesium. During the one-year fellow-up, serum potassium and magnesium was sustained in normal range.

Biochemistry profiles of the other family pedigree

One of his sisters (II-4, Fig. 2A) also showed hypokalemia, hypomagnesaemia, elevated renin-aldosterone level, normal blood pressure, but with normocalcemia and hypocalciuria (Table 1). The sister was administrated with potassium chloride, and the serum potassium level was corrected into normal range, without symptoms such as fatigue, muscle weakness, tetany, or paresthesia during the treatment. In addition, the levels of serum potassium, sodium, calcium, magnesium, urinary potassium and calcium were unremarkable in the other family members of the pedigree (Table 2).
Table 2
Biochemical data of the GS pedigree.

| Age/Sex | I-1  | I-2  | II-1 | II-2 | II-4 | II-5 | II-6 (proband) | Normal range |
|---------|------|------|------|------|------|------|----------------|--------------|
|         | 79M  | 79F  | 55F  | 53M  | 48F  | 47F  | 42M            |              |
| Biochemistry profile |      |      |      |      |      |      |                |              |
| Serum K (mmol/L) | 3.94 | 4.19 | 4.35 | 4.01 | 3.08 | 4.13 | 1.9–2.8 ↓↓    | 3.5–5.5      |
| Serum Mg (mmol/L) | 0.99 | 0.85 | 0.99 | 0.95 | 0.68 | 0.97 | 0.71 ↓         | 0.8–1.02     |
| Serum Ca (mmol/L) | 2.36 | 2.31 | 2.47 | 2.37 | 2.5  | 2.32 | 2.48–2.6 ↑     | 2.1–2.52     |
| FE K (%) | 6.56 | 6.33 | 5.56 | 8.12 | 12.6 | 6.38 | 30.5–49.2 ↑↑   | 8–12%        |
| uCa/Cr  | 0.29 | 0.30 | 0.34 | 0.19 | 0.03 | 0.28 | 0.7 ↑          | <0.2         |
| FE Ca (%) | 0.46 | 0.73 | 0.61 | 0.42 | 0.05 | 0.51 | 2.66 ↑         | <1%          |

Genetic analysis

The amplification and sequencing of SLC12A3 gene were performed on the family pedigree. The genetic analysis revealed that the proband and his sister (II-4) had same compound missense mutations in Exon 3, 8, 13, and genotypes were c.433 C > T (p.Arg145Cys, Het), c.1077 C > G (p.Asn359Lys, Het), and c.1666 C > T (p.Pro556Ser, Het). His father (I-1) carried c.1077 C > G (p.Asn359Lys, Het), his mother (I-2) and the other siblings (II-1, II-2, II-5) carried c.433 C > T (p.Arg145Cys, Het) and c.1666 C > T (p.Pro556Ser, Het), but they were phenotypically healthy (Fig. 2, Supplement figure, Table 2–3). To our knowledge, the compound heterozygous mutations c.1077 C > G (p.Asn359Lys) and c.1666 C > T (p.Pro556Ser) were novel. We did not detect mutations reported to be associated with BS, including CLCNKA/CLCNKB (encodes the chloride channel CIC-Kb) and BSND (encodes chloride channel accessory subunit) or KCNJ1 (encodes the thick ascending limb potassium channel).

Table 3
Summary of the variants of SLC12A3 in the GS pedigree.

|         | Nucleotide mutations | Amino acid variants | Predicted effect | exon |
|---------|----------------------|---------------------|------------------|------|
| I-1     | c.433 C > T          | p.Arg145Cys         | Het              | 3    |
| I-2     | c.1077 C > G         | p.Asn359Lys         | Het              | 8    |
| I-3     | c.1666 C > T         | p.Pro556Ser         | Het              | 13   |
| II-6    | c.433 C > T          | p.Arg145Cys         | Het              | 3    |
| II-3    | c.1077 C > G         | p.Asn359Lys         | Het              | 8    |
| II-1    | c.1666 C > T         | p.Pro556Ser         | Het              | 13   |

Three-dimensional structure prediction of NCCT and the potential dysfunction
The SLC12A3 encoded NCCT contains 12 transmembrane segments and N- and C-terminal domains. We identified the alteration of NCCT structure induced by the variants of SLC12A3 (C433T, Arg145Cys; C1077G, Asn359Lys; and C1666T, Pro556Ser) (Fig. 3), using the SWISS-MODEL workspace (http://swiss-model.expasy.org). Those missense mutations led to the predicted amino acid change, caused differences of NCCT protein structures and function, finally leading to the electrolyte disturbance.

In this study, we reported a family pedigree of GS with novel heterozygous mutations of SLC12A3, exhibiting hypokalemia and hypomagnesemia. However, the proband exhibited hypercalciuria and renal calcification, which made it difficult to differ from BS. The persistent potassium excretion resulted in renal tubular lesions such as vacuolar degeneration, and affected urinary calcium reabsorption and calcium excretion. With administration of aldosterone antagonist, potassium and magnesium supplement, the serum potassium was maintained in nearly normal range, without hypokalemia associated symptoms. We reported the novel c.1077 C > G (p.Asn359Lys) and c.1666 C > T (p.Pro556Ser) and the contribution to clinical features of GS. Genetic analysis is a useful tool for the diagnosis and differential diagnosis. The phenotype variability may be associated with the pathogenic variabilities of SLC12A3 mutations. Further investigation is needed to provide better understanding of genotype-phenotype association of NCCT dysfunction in GS.

Discussion
GS is a salt-losing tubulopathy with the clinical features of hypokalemic alkalosis, hypomagnesaemia and hypocalciuria. Chronic low potassium leads to symptoms of weakness, fatigue, thirst and the abnormal heart palpitation. Severe case can cause rhabdomyolysis, ventricular arrhythmias, or sudden cardiac arrest [9]. GS is associated with dysfunction of NCCT encoded by SLC12A3 gene in the renal distal convoluted tubule (DCT). The decreased reabsorption of Na\(^+\) and Cl\(^-\) leads to compensatory excessive exchange through Na\(^+\)/K\(^+\) and Na\(^+\)/H\(^+\) pump, and results in excessive K\(^+\) and H\(^+\) excretion and hypokalemic alkalosis. In a small minority of GS patients, mutations in the CLCNKB gene encoding the chloride channel CIC-Kb have been identified [10].

To our knowledge, this is the first time to report the novel c.1077 C > G (p.Asn359Lys) and c.1666 C >
T (p.Pro556Ser) mutations of SLC12A3 and its relation with phenotypes. The phenotypes are more severe in patients with more than one mutated alleles, with lower serum potassium level, and more difficult to be corrected with potassium supplement [6, 11].

BS (especially type III) is the most important renal salt-wasting disease which should be considered as the differential diagnosis of GS. BS is also characterized by hypokalemia, metabolic alkalosis, polyuria, increased renin activity and aldosterone level, but without hypertension or edema. It exhibits the increased urinary calcium excretion, but rarely leads to nephrocalcinosis. BS is caused by mutations of NKCC2 (Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter) in the thick ascending limb (TAL) of Henle loop (Type 1 BS) [12], ROMK (outwardly rectifying potassium channel) (Type 2 BS), or CLCNKB (chloride channel) (Type 3 BS) which is a regulator of NKCC2. A minority of GS patients has been shown to have mutations of CLCNKB gene [10]. Type 4 BS is induced by mutations of both the kidney-specific chloride channel CIC-Ka and CIC-Kb, leading to dysfunction of Cl\(^{-}\) reabsorption. Activating mutations of calcium-sensing receptor (CaSR) suppresses the NKCC2 and ROMK expression to induce type 5 GS [13]. The site of defect in BS is at the TAL of the Henle loop, whereas in GS is at the renal DCT [14].

GS used to be thought as a mild type of BS. However, the pathogenesis and clinical characteristics are different. BS typically presents in infancy or early childhood, with more severe clinical manifestations and complications, such as severe electrolyte derangements, short stature, polyuria, and hypercalciuria induced nephrocalcinosis [15]. GS usually shows hypomagnesaemia with increased urinary manganese excretion (FEMg > 4%), but lower urinary calcium excretion (uCa/uCr < 0.2) [8].

Diuretic loading test using furosemide and hydrochlorothiazide is helpful to differ GS from BS [16]. Usually, hypocalciuria in GS is related to the increased calcium reabsorption in the proximal tubule and distal renal unit, which is caused by NCCT dysfunction [17]. In this case, the proband exhibited hypokalemia, hypomagnesaemia, metabolic alkalosis, but with hypercalcuria, similar to the features of BS, which makes it confused for differential diagnosis. It is contradicted with the features of hypocalciuria in classic GS. Chronic renal potassium loss can cause renal tubular epithelial cell injury or vacuolar deformation, to reduce the reabsorption of calcium [18]. In addition, loss-of-function of
NCCT up-regulates the expression of intestinal calcium transporter, and increases calcium uptake in gut tract [19]. Hypercalcemia inhibits PTH release by negative feedback. In reverse, the suppressed PTH level reduces the calcium reabsorption by the renal tubule, and increases urinary calcium excretion. This patient also has diabetes mellitus. Hyperglycemia causes osmotic diuresis to increase urinary calcium excretion. Increased urinary calcium excretion and chronic hypomagnesaemia are the causes of renal calcification. The relationship between mutated gene sites and urinary calcium levels has not been reported. It is unclear whether hypercalcuria is associated with three mutations of SLC12A3.

It is reported that GS patient have a tendency of glucose intolerance and impaired insulin secretion [20]. Potassium plays an important role in the regulation of insulin release. Reduced extracellular potassium ion concentration could suppress the insulin secretion and release via ATP sensitive potassium channel on beta cells. Long-term low potassium and magnesium level is one of the factors of diabetes development. In addition, hyperaldosteronism was also reported to promote insulin resistance [21].

Studies have shown that GS can be combined with autoimmune diseases, such as Graves' disease, Hashimoto's thyroiditis, IgA nephropathy, Sjogren's syndrome, or latent autoimmune diabetes in adults (LADA) [22, 23].

The therapeutic strategy of GS focuses on the correction of electrolyte disturbance, especially potassium and magnesium replacement. The level of serum magnesium may affect the severity and effect of potassium supplement [6, 24]. Other options include the inhibitors for the secondary elevated renin-aldosterone system (RAAS), such as non-selective or selective aldosterone antagonist antisterone or eplerenone, or NaCl transporter blockers such as aminophenidine [25]. Non-steroidal anti-inflammatory drugs (NASID) such as indomethacin can suppress renin secretion by inhibiting renal prostaglandin E2 (PGE2) synthesis, and ameliorate the up-regulation of aldosterone level induced by potassium supplement. It also could increase potassium level without worsening sodium and volume depletion in GS patients [26]. However, the gastrointestinal side effect and interstitial renal damage make the application to be limited.
Conclusions
In this study, we identified novel mutations of SLC12A3 and reviewed the advances in genetic analysis, diagnosis, differential diagnosis and management of GS. Combined with clinical features, biochemistry profiles and genetic analysis, the diagnose could be made. Further studies on the correlation between genotype and phenotype are needed to provide better understanding of GS.

List Of Abbreviations
GS, Gitelman's syndrome
NCCT, the Na-Cl co-transporter
RAAS, renin-angiotensin-aldosterone system
BS, Bartter's syndrome
PCR, polymerase chain reactions
NGS, next generation sequencing
FEK, fraction excretion rate of potassium
ACTH, adreno-corticotropic hormone
PTH, parathyroid hormone
GSIS, glucose stimulated insulin secretion
CT, computed tomography
DCT, distal convoluted tubule
NKCC2, Na+-K+-2Cl- cotransporter
TAL, thick ascending limb
CaSR, calcium-sensing receptor
LADA, latent autoimmune diabetes in adults
ANA, anti-nuclear antibodies
ENA, extractable nuclear antigen
NASID, Non-steroidal anti-inflammatory drugs
PGE2, prostaglandin E2

Declarations
Ethic approval and consent to participate:
All procedures performed in this study involving human participants were in accordance with the ethical standards of the Affiliated Hospital of Qingdao University with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. We obtained the written informed consent from all the participants.

**Consent to publish:**

The clinical data and images were obtained from the proband and his family members. All the participants gave their written consent for the information to be published.

**Availability of data and materials:**

The data generated and analyzed in this study are not publicly available due to protection of privacy, but are available from the corresponding author on reasonable request.

**Competing interests:**

All authors declare that they have no competing of interest.

**Funding:**

This work was supported by the grant from the National Natural Science Foundation of China (Grant No. 81600691) and Shandong Provincial Natural Science Foundation, China (Grant No. ZR2016HB08) for clinical data collection, including biochemistry profiles and genetic analysis.

**Authors’ contributions:**

DB and CY collected and interpreted the patient and family pedigree’ data, analyzed the clinical data and genetic sequencing, summarized and wrote the paper. LX is the major contributors in writing the manuscript. WY provided key guidance of intellectual content and important discussion to this study. WF, ZY, and SX provided important discussion and suggestions. ZW summarized all results and controlled the whole study. All authors read and approved the final manuscript.

**Acknowledgements:**

We are grateful to the patient and his family members for their participation in this study. We thank Prof. Li Yujun and Dr. Shao Shihong (Department of pathology, the Affiliated Hospital of Qingdao University) performed the histological examination of the kidney. We also thank Prof. Shao Leping (Department of nephrology, the Qingdao Municipal Hospital) for the suggestion and discussion. All
authors have no conflict of interest related to this work.

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Tables

| II-4 | II-6 (proband) | Normal range |
|------|----------------|--------------|

Table 1. Biochemical profiles of the proband and his sister diagnosed with GS.
| Age/Sex     | 48F | 42M |
|------------|-----|-----|
| **Biochemistry** |     |     |
| Serum K (mmol/L) | 3.08↓ | 1.9-2.8↓ | 3.5-5.5↓ |
| Serum Mg (mmol/L) | 0.68↓ | 0.71↓ | 0.8-1.02↓ |
| Serum Ca (mmol/L) | 2.5 | 2.48-2.6 | 2.1-2.52 |
| FE K (%) | 12.66 | 30.5-49.2↑↑ | 8-12% |
| uCa/Cr | 0.03-0.07↓↓ | 0.7↑ | <0.2 |
| FE Ca (%) | 0.05-0.14↓↓ | 2.66↑ | <1% |
| PTH (pg/mL) | 32.57 | 5.69 | 15-65 |
| Fasting blood glucose (mmol/L) | 5.60 | 7.42 |
| OGTT-2h BG (mmol/L) | - | 11.2 |
| Test Description                            | Value 1 | Value 2 | Reference Range |
|--------------------------------------------|---------|---------|-----------------|
| Fasting C-peptide (ng/mL)                  | -       | 4.07    |                 |
| OGTT-2h C-peptide (peak)                   | -       | 15.41   |                 |
| RAAS (lying condition)                     |         |         |                 |
| Renin (ng/mL/hr)                           | 2.47    | 3.58    | 0.15-2.33       |
| Aldosterone (pg/mL)                        | 79.53   | 123.72  | 30-160          |
| RAAS (standing condition)                  |         |         |                 |
| Renin                                      | 9.36    | >13.56  | 0.10-6.56       |
| Aldosterone                                | 143.5   | 279.65  | 70-300          |
| Arterial blood gas                         |         |         |                 |
| pH                                         | 7.38    | 7.4     | 7.35-7.45       |
Table 2. Biochemical data of the GS pedigree.
| Test       | Units | Lower Limit | Upper Limit | Result   | Reference Range |
|------------|-------|-------------|-------------|----------|-----------------|
| Serum K    | mmol/L|             |             | 4.13     | 1.9-2.8         |
| Serum Mg   | mmol/L|             |             | 0.97     | 0.71-1.0       |
| Serum Ca   | mmol/L|             |             | 2.32     | 2.48-2.6       |
| FE K       | %     |             |             | 6.38     | 30.5-90        |
| uCa/Cr     |       |             |             | 0.28     | 0.7           |
Table 3. Summary of the variants of *SLC12A3* in the GS pedigree.
| Nucleotide mutations | Amino acid variants | Predicted effect | Exon |
|----------------------|---------------------|-----------------|------|
| ① Proband (I-6)     | c.433 C>T          | p.Arg145Cys     | Het  |
| Sister (II-3)        | c.1077 C>G         | p.Asn359Lys     | Het  |
|                      | c.1666 C>T         | p.Pro556Ser     | Het  |
| ② Father (I-1)      | c.1077 C>G         | p.Asn359Lys     | Het  |
|                      |                     |                 |      |
| ③ Mother (I-2)      | c.433 C>T          | p.Arg145Cys     | Het  |
| Other siblings       | c.1666 C>T         | p.Pro556Ser     | Het  |

Figures
Figure 1

Imaging manifestations and renal biopsy to show renal lesions in the GS patient. A-D. Computed tomography (CT) scan of the patient. Arrows to show renal calcification. C and D are the magnification of A and B, respectively. E-H. Renal pathomorphism of the patient to show renal tubular lesions. E. Hematoxylin-eosin (HE) staining. F. Periodic acid Schiff (PAS) staining. G. Sliver methenamine (SM) staining. H. Congo red staining. Those show renal tubular atrophy, epithelial cell edema, and the thickening of basal membrane. The vacuolar degeneration of tubular epithelial cells and loss of brush border were observed. SM and Congo red staining were negative. (x200) Arrowheads to show degenerated tubular epithelial cells.
Genetic analysis of SLC12A3 mutations in the pedigree of Gitelman’s syndrome. A. Pedigree for the family structure. Marked symbols to show patients with clinical manifestations induced by compound heterozygous mutations of SLC12A3. Mutations of c.433 C>T and c.1666 C>T was presented as black, and c.1077 C>G was showed as grey. Circles present females, and squares present males. Arrow shows proband. The III-1 and III-2 show normal phenotypes, without characterists of GS. However, the genetic analysis was not performed on them. B. Sequencing results of variants of SLC12A3. The patient (II-6, proband) and his mother (I-2), brother (II-2) and sisters (II-1; II-4; II-5) carried heterozygous mutation of C433T (Arg145Cys) and C1666T (Pro556Ser) in Exon 3 and 13 of SLC12A3, respectively. Heterozygous mutation of C1077G (Asn359Lys) in Exon 8 was detected in the patient (II-6, proband), his father (I-1), and the sister with GS (II-4). Arrows indicate heterozygous nucleotide substitutions.
The model structure of Na-Cl cotransporter (NCCT) protein with variants induced by novel mutations of SLC12A3 to show potential influence. The differences of modeled structure compared to wild type (A) were indicated in cycles. The visible differences of protein structure was induced by (B) c.433 C>T (p.Arg145Cys) and c.1666 C>T (p.Pro556Ser), or (C) c.1077 C>G (p.Asn359Lys). Co-existence of c.433 C>T (p.Arg145Cys), c.1077 C>G (p.Asn359Lys) and c.1666 C>T (p.Pro556Ser) lead to differences from wild type protein structure. It may induce the alteration of the function of NCCT.

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