Hypokalemia, hypomagnesemia, hypocalciuria, and recurrent tetany: Gitelman syndrome in a Chinese pedigree and literature review

Ming-Feng Xia¹,†, Hua Bian¹,†, Hong Liu², Hui-Juan Wu³, Zhi-Gang Zhang³, Zhi-Qiang Lu¹ & Xin Gao¹

¹Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, Shanghai, China
²Department of Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China
³Department of Pathology, School of Basic Medical Sciences, Fudan University, Shanghai, China

Correspondence
Xin Gao, Department of Endocrinology and Metabolism, Zhongshan Hospital, Fudan University, No. 180 Fenglin Road, Shanghai, China Shanghai, China. Tel: +86 21 64041990-8021; Fax: +86 21 64037269; E-mail: zhongshan_endo@126.com

Key Clinical Message
Gitelman syndrome is an autosomal recessive disease mostly associated with loss-of-function mutations of the SLC12A3 gene and featured by clinical hypokalemia, hypomagnesemia, hypocalciuria, and histologically hypertrophy of the juxtaglomerular apparatus. A novel homozygous mutation (p.Arg399Pro) at the extracellular domain of SLC12A3 was found and correlated with the severe clinical manifestations.

Keywords
Gitelman syndrome, phenotype–genotype relation, review, SLC12A3.

Introduction
Gitelman syndrome (GS; Mendelian Inheritance in Man 263800) is an autosomal recessive inherited disease mostly associated with loss-of-function mutations of the SLC12A3 (solute carrier family 12 member 3) gene [1], which encoded the thiazide-sensitive sodium chloride co-transporter (NCCT) of the distal convoluted tubule (DCT) [2]. The prevalence of GS is approximately 1% as heterozygotes and 1 in 40,000 as homozygotes [2]. Clinical symptoms for this disease are hypokalemia, hypomagnesemia, hypocalciuria, and hypochloremic metabolic alkalosis [3]. At present, hypokalemia is a common clinical disorder in approximately 20% of hospital inpatients [4], and GS must be considered as a differential diagnosis of some settings of clinical hypokalemia.

The pathogenic gene of GS, SLC12A3 gene (GeneID: 6559; MIM: 600968; GeneBank: NC_000016.10) is located on human chromosome 16q13 and consists of 26 separate exons [5]. There have been over 425 SLC12A3 mutations found to be associated with GS [6, 7] using human genome database search (HGMD, http://www.hgmd.org) and may
show a considerable phenotypical variability clinically [8]. Most patients with GS were asymptomatic or mild [9], but a few may show an early onset, severe neuromuscular manifestations (e.g., tetany, seizures, and rhabdomyolysis) [10]. The potential explanations for the phenotypical variability in GS may include genetic heterogeneity, position, and nature of mutations [11]. However, the correlation between the type and position of \( SLC12A3 \) gene mutation and the severity of clinical symptoms in GS has not been fully deciphered yet. Therefore, identification of novel mutation and its associated clinical manifestations will provide further insights into the functional domains of NCCT and the genotype–phenotype correlations in GS patients, which may lead to personalized treatment.

In this study, we report a Chinese patient with a homozygous p.Arg399Pro mutation in the \( SLC12A3 \) gene, who had severe clinical manifestations and typical pathological features of GS. We also review the literature, provide an outline of \( SLC12A3 \) mutations in Chinese population, and analyze the associations of \( SLC12A3 \) mutation types and sites with clinical manifestations.

**Case Report**

**Clinical data of proband and other family members**

A 29-year-old Chinese woman was referred to our Endocrinology unit with a 10-year history of fatigue, recurrent tetany, and aggravation of these symptoms for 1 h. She once visited the local hospital and was found to have hypokalemia, but further diagnosis was not made for the cause of hypokalemia. She felt better after supplementation of potassium chloride, but still experienced recurrence and aggravation of symptoms after that. She had frequent cramps on her face, and physical examination revealed a sitting blood pressure of 110/70 mmHg. She did not have a history of long-term use of laxatives, diuretics, ethanol, or drug addiction nor a history of hypertension. Her parents were nonconsanguineous, and her family members were all asymptomatic.

**Laboratory findings**

The laboratory findings revealed severe hypokalemia (1.9 mmol/L, reference: 3.5–5.3 mmol/L), hypomagnesemia (0.45 mmol/L, reference: 0.67–1.04 mmol/L), metabolic alkalosis (PH 7.55 and plasma bicarbonate 36 mmol/L, reference: 23–29 mmol/L), hyperkaliuria (57.7 mmol/24 h), and hypocalciuria (urine calcium/creatinine ratio = 0.023, reference: 0.05–0.57 mmol/mmolCRE). The plasma aldosterone (216 pg/mL, reference: 59.5–173.9 pg/mL) and renin activity (2.4 ng/mL/h, reference: 0.05–0.79 ng/mL/h) were increased, and the creatine kinase was extremely high (25,355 U/L, reference: 34–174 U/L) on admission.

**Genotype analysis**

The patients and her family members were screened for possible mutations associated with urinary diseases,
including SLC12A3 and CLCNKB genes, by next-genera-
tion sequencing (NGS). Sanger sequencing was used to
confirm the detected mutation in the proband. The
details of mutation analysis were shown in Appendix S1.
Sequence analysis of the SLC12A3 gene revealed a
homozygous mutation in exon10 (c.1196G>C) (Fig. 1A).
The molecular structures of the normal and mutant
SLC12A3 proteins were modeled using the SWISS-
MODEL protein structure modeling server (www.swiss
model.expasy.org) [12]. This change is predicted to sub-
stitute the hydrophilic amino acid arginine at codon 399
by a hydrophobic amino acid proline, which is located
at the extracellular region of NCCT protein. The pre-
dicted molecular structure of the wild-type SLC12A3
and p.R399P protein was shown in Figure 1B. The
mutation was also found at a single heterozygous state
in her parents and two daughters (Fig. 1C).

Renal histological examination
To determine the effect of SLC12A3 p.R399P mutation
on the kidney histology, a renal biopsy was performed
10 days after correction of serum creatine kinase level
(Fig. 2). Renal tissue was obtained through percuta-
neous needle biopsy performed at department of
nephrology, Zhongshan Hospital (Shanghai, China). The
tissue sample was processed for light microscopy
and electron microscopy examinations according to the stan-
dard protocols of Department of Pathology, School of
Basic Medical Sciences, Fudan University (Shanghai,
China). Light microscopy showed hypertrophy of the
juxtaglomerular apparatus with proliferation of extra-
glomerular mesangial cells with normal morphology of
glomeruli (Fig. 2A). Proliferation of extraglomerular
mesangial cells was also found under electron micro-
scopy (Fig. 2B), and there were many secretive granules
in the cytoplasm of extraglomerular mesangial cells
(Fig. 2C and D).

Treatment and follow-up
After the clinical diagnosis was confirmed, the patient
received the following treatment: potassium chlo-
ride sustained release tablets 1 g, potassium–magnesium
aspartate oral liquid 10 mL and spironolactone 80 mg,
taken orally three times daily. After the treatment, the
symptoms improved, the serum potassium and magne-
sium increased to the range of 3.2–3.5 mmol/L and
0.63–0.83 mmol/L, and serum creatine kinase level
recovered within the normal range. Severe hypokalemia
has not recurred during a one-year follow-up after dis-
charge.

Correlation between phenotype and
genotype
To find the possible explanation for the severe clinical
manifestations of the GS patient in our current study, the
data of all Chinese GS patients in previous publications,
which could be found in the PubMed database to date,
were collected to analyze the associations between the
types and sites of SLC12A3 gene mutations and severity
of clinical manifestations in GS. As shown in Table 1, 252
patients (157 male and 95 female) with GS have been
described in the literature [6, 13–25]. The average age at
onset was 23.6, and hypomagnesemia and hypocalciuria
were found in 92% and 88% of GS patients (Table 1).
Patients with early onset of symptoms had significantly
lower serum potassium level ($r = 0.561$, $P = 0.029$), and
the serum magnesium level was positively associated with
serum potassium level ($r = 0.625$, $P = 0.013$) by Pearson’s
correlation analyses (Fig. 3). The mutations were dis-
tributed throughout the whole SLC12A3 gene. To analyze
the correlation between the types and sites of SLC12A3
mutations and clinical manifestations, we divided the
patients of GS into groups of single heterozygotes, com-
plex heterozygotes, and homozygotes, and the mutated
alleles were classified as intracellular, transmembranal,
and extracellular mutations according to their sites. Com-
plex heterozygotes/homozygotes of SLC12A3 gene muta-
tions had significantly lower serum potassium than the
single heterozygotes, and the patients with SLC12A3
mutations extracellularly also had significantly lower
serum potassium and magnesium levels than those with
mutations transmembranally or intracellularly (Table 2).

Discussion
Gitelman et al. described the clinical features of GS for
the first time in 1966 [1]. In 1996, Simon et al. cloned
cDNA of SLC12A3 and indentified SLC12A3 gene muta-
tion as the cause of GS [2]. NCCT is coded by SLC12A3
gene and constituted by 1021 amino acid residue, which
predict 12 membrane spanning domain and longer amino
and carboxyl end within the cells. In our current study,
we reported a GS patient with a novel homozygous muta-
tion in the SLC12A3 gene (c.1196G>C) that subsequently
led to substitution of the basic amino acid arginine with
a hydrophobic amino acid proline at the extracellular
region of NCCT protein. Phenotypically, this patient had
severe GS symptoms of hypokalemia, hypomagnesemia,
hypocalciuria, and recurrent onsets of tetany. We also
found that the severe clinical manifestations of our
patients might be associated with its homozygous muta-
tion at the NCCT extracellular region by analyzing the
relationships between clinical manifestations and SLC12A3 mutation types and sites in all published studies of GS patients in China.

Sodium chloride co-transporter participates in the control of ion homeostasis at the distal convoluted tubule portion of the nephron. Loss-of-function mutations in NCCT will impede the reabsorption of sodium in the DCT, and result in more sodium arriving at the collecting duct and mild volume contraction. To maintain the salt homeostasis, the exchange between Na⁺/K⁺, H⁺/K⁺, and

Figure 2. Renal biopsy of the case presenting with Gitelman syndrome. (A) Light microscopic examination showed hypertrophy of juxtaglomerular apparatus (more than 50% of glomeruli involved) with proliferation of extraglomerular mesangial cell. The morphology of glomeruli is almost normal. (B) Electron microscopy revealed proliferation of extraglomerular mesangial cells. (C, D) Numerous secretive granules in the cytoplasm of this extraglomerular mesangial cell.
| Author, year | Location | Mutation | Consequence |
|-------------|----------|----------|-------------|
| Zha (2015) [13] | SH | c.791C | p.G264A |
| Luo (2015) [6] | FJ | c.2782T, c.2129A | p.R928C, p.S710X |
| Lu (2015) [14] | SC | c.487T | p.T163M |
| Li (2015) [15] | SD | c.179T, c.234delG, c.1925A, c.486-490DelTACGGinsA | p.T660M, p.R642H, p.162frameshift |
| Jiang (2015) [16] | BJ | Unknown | p.G439S, p.S615L, p.R913Q, p.114Frameshift, p.T60M, p.R919C, p.Y386C, p.K545L, p.G800W |
| Jiang (2014) [17] | BJ | Unknown | p.R655H, p.T60M, p.N566L, p.R913Q, p.556Frameshift |
| Ren (2013) [18] | SH | c.181T, c.1294G, c.1322T, c.346-353delACTGATGG, c.1718G, c.2969insGCT, c.1083G, c.1322T, c.2717A, c.2129A, c.1113G, Dei n7426-n7438, Ins (acccgaaaatttt), c.1693C, c.1462A, c.2404T | p.G64A, p.R928C, p.S710X, p.A370P, p.G800R, p.Q131K, p.G201D, p.V169I, p.L170Q, p.Y70C, p.R861C, p.L215P, p.W844T, p.809Fframeshift, p.R913Q, p.V677M, p.S976F, p.T660M, p.L700V, p.T428I, p.G196V, p.959frameshift |
| Tseng (2012) [19] | TW | Unknown | p.A13P, p.D62G, p.T60M, p.R83Q, p.H90Y, p.R145C, p.T163M, p.L215P, p.H234Q, p.S283Y, p.114Frameshift, p.G439V, p.959frameshift, p.L571P, p.997mcC, p.R928C, p.N359L, p.R913Q, splice mutation, p.S710X, p.R919C, p.Y386C, p.K545L, p.G800W |
| Sung (2011) [20] | TW | Unknown | p.A13P, p.D62G, p.T60M, p.R83Q, p.H90Y, p.R145C, p.T163M, p.L215P, p.H234Q, p.S283Y, p.114Frameshift, p.G439V, p.959frameshift, p.L571P, p.997mcC, p.R928C, p.N359L, p.R913Q, splice mutation, p.S710X, p.R919C, p.Y386C, p.K545L, p.G800W |
| Lo (2011) [21] | TW | Unknown | p.T163M, p.T649M, p.688Frameshift, p.frameshift, p.L215P, p.R83Q, p.T163M, p.T660M, p.R871H, p.W844X, p.R642C |
| Qin (2009) [22] | SH | c.181T, c.2761T, c.1462A, 492_496delTACGGinsA, c.1022T, c.1083G, c.1322T, c.2717A, IVS7-1 G > A, g.7427_7438delinsCCGAAAATTTT, c.2717A, IVS16-2 A > G, c.1268T, c.1970A, c.1113G | p.T163M, p.T649M, p.688Frameshift, p.frameshift, p.L215P, p.R83Q, p.T163M, p.T660M, p.R871H, p.W844X, p.R642C |
| Sung (2011) [20] | TW | Unknown | p.T163M, p.T649M, p.688Frameshift, p.frameshift, p.L215P, p.R83Q, p.T163M, p.T660M, p.R871H, p.W844X, p.R642C |
| Lo (2011) [21] | TW | Unknown | p.T163M, p.T649M, p.688Frameshift, p.frameshift, p.L215P, p.R83Q, p.T163M, p.T660M, p.R871H, p.W844X, p.R642C |
| Qin (2009) [22] | SH | c.181T, c.2761T, c.1462A, 492_496delTACGGinsA, c.1022T, c.1083G, c.1322T, c.2717A, IVS7-1 G > A, g.7427_7438delinsCCGAAAATTTT, c.2717A, IVS16-2 A > G, c.1268T, c.1970A, c.1113G | p.T60M, p.R919C, p.Y386C, p.K545L, p.G800W, p.162frameshift, p.T339I, p.N359L, p.G439V, p.R904Q, splice mutation, p.C421F, p.R655H, p.Y386C |

(Continued)
Na+/H+ was increased in the cortical collecting duct at the expense of increased secretion of potassium and hydrogen ions, which led to hypokalemia and metabolic alkalosis. The low volume also activates the renin–angiotensin–aldosterone system, which stimulates the proliferation of juxtaglomerular apparatus and increases the renin activity and aldosterone levels in GS. On the other hand, the passive Ca²⁺ reabsorption in the proximal tubule and reduced abundance of the Mg²⁺ channel TRPM6 in the DCT explains hypocalciuria and hypomagnesemia, respectively [26].

Pathologically, the patient was characterized by hypertrophy of the juxtaglomerular apparatus, and we also found many secretive granules in the cytoplasm of extraglomerular mesangial cells under electron microscopy, which conformed to the typical pathologic manifestations of GS. Although hypokalemia-induced rhabdomyolysis was suspected in our patient, her serum creatine level was normal and no renal tubular necrosis was found under light microscope 10 days after correction of serum creatine kinase level. Therefore, it was not likely that the renal pathological change was affected by the rhabdomyolysis.

To date, more than 100 mutations have been reported in Chinese GS patients (Table 1). All patients had hypokalemia and 92% had hypomagnesemia. Our analysis based on the previous reports showed that GS patients with lower potassium were more symptomatic, and usually had a younger age at diagnosis and lower serum magnesium level. It has been reported the co-localization of NCCT and TRPM6 proteins [17], which might indicate the functional status of NCCT might regulate the Mg²⁺ channel TRPM6.

Traditionally, the GS is recessively inherited, with simple heterozygous relatives being asymptomatic. However, there is still a proportion (13.1%) of affected individuals with only one SLC12A3 mutant allele detectable in the Chinese GS patients. Single heterozygotes of patients with GS have been reported previously [27]. Although a second mutation in some nonstudied region or in other genes cannot be excluded, the patients with SLC12A3 single heterozygous mutation showed a milder manifestation of hypokalemia in comparison with the complex heterozygotes or homozygotes in the current study (Table 2). It has been reported that one heterozygous mutation in SLC12A3 gene would partially impair the renal function for salt handing [28]. Also, the expression of NCCT may be influenced by epigenetic modifications and/or silent polymorphisms, which lead to impaired function in simple heterozygotes [29]. Therefore, it is still necessary to screen for potential hypokalemia in the subjects carrying single heterozygous SLC12A3 gene mutations.

Previous studies have shown that the SLC12A3 mutations scattered through the whole coding sequence of the

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**Table 1. Continued.**

| Author, year | Location | Mutation | Consequence | Male | Female | Age at onset | K+, mmol/L | Mg²⁺, mmol/L | Hypocalciuria | Hypomagnesemia |
|--------------|----------|----------|-------------|------|--------|-------------|----------|-------------|-------------|---------------|
| Miao (2009) [23] | SH | c.G593T, c.G1322T, c.C185T, c.T1294G, c.1384delG, c.G1462A, c.346-353delACTGATGG | p.G196V, p.G439V, p.T60M, p.C430G, p.114frameshift, p.460frameshift, p.959frameshift, p.L571P, p.997insC, p.D486N | 9/4 | 26 | 2.5 | 0.52 | 12/1 | 12/1 |
| Shao (2008) [24] | SH | c.346-353delACTGATGG | p.959frameshift | 3/2 | 15 | 2.36 | 0.53 | 3/2 | 5/0 |

SH, Shanghai; FJ, Fujian; SC, Sichuan; SD, Shandong; BJ, Beijing; TW, Taiwan.
NCCT protein, but most of the mutations are frequently found in the intracellular domains of the protein [30], and the phenotype of GS is highly heterogeneous [11]. Jiang et al. found the percentage of mutated alleles distributed extracellularly was greater in hypo- than normomagnesemic patients [17]. In our review of Chinese GS, most of the SLC12A3 mutation alleles were located on the intracellular domains of NCCT, and we found the average serum potassium and magnesium was significantly lower in subjects with SLC12A3 mutations extracellularly than those with mutations intracellularly or transmembranally (Table 2). Different domains of the NCCT protein have been found to have different functions [31]. In general, the basic structure of the Na+-coupled chloride cotransporters features a central hydrophobic domain containing 12 α-helices that is flanked by a short hydrophilic amino-terminal domain and a long predominantly hydrophilic carboxy-terminal domain within the cell [32]. There is a long hydrophilic loop connecting transmembrane segments 7 and 8, exhibiting three putative N-glycosylation sites in extracellular domain of NCCT, which is distinguished with other electroneutral cation-chloride cotransporters, like the K+-coupled chloride cotransporters (Fig. S1) [29]. Interestingly, the p.R399P SLC12A3 mutation of the proband in our study was located at one end of the extracellular long hydrophilic loop of NCCT, and one previous functional study in Xenopus oocytes had shown that NCCT with R399C mutants almost lost the whole function of Na+ uptake [29]. Therefore, the severe clinical manifestation of the patient in our current study might be related to its mutation site at the extracellular long hydrophilic loop of NCCT.

Gitelman syndrome is a rare inherited disease, and genetic diagnosis is not commonly used in clinical practice, so it is difficult to enroll sufficient GS patients for phenotype–genotype correlation analysis in one single clinical center. Therefore, we collected the data of all GS

![Figure 3. Associations of serum potassium with age at onset (panel on the left) and serum magnesium (panel on the right). The sizes of bubbles represent the sample sizes of different studies.](image)

| SLC12A3 gene mutation type | SLC12A3 gene mutation sitea | Number | Single heterozygotes | Complex heterozygotes | Homozygotes | P for trend | Intracellular mutations | Transmembranal mutations | Extracellular mutations | P for trend |
|---------------------------|---------------------------|--------|---------------------|----------------------|-------------|------------|-----------------------|-------------------------|------------------------|------------|
| Age                       |                           | 58     | 27 (19–34)          | 25 (17–37)           | 32 (21–39)  | 0.177      | 27 (18–37)           | 20 (15–37)              | 28 (19–34)             | 0.679      |
| Serum potassium, mmol/L   |                           | 2.75 ± 0.33 | 2.34 ± 0.46*       | 2.28 ± 0.52*        | 0.029      | 2.37 ± 0.47 | 2.32 ± 0.55          | 2.07 ± 0.16†            | 0.275      |
| Serum magnesium, mmol/L   |                           | 0.55 ± 0.11 | 0.56 ± 0.15         | 0.61 ± 0.18         | 0.183      | 0.59 ± 0.14 | 0.57 ± 0.04          | 0.44 ± 0.08‡†          | 0.021      |

*A total of 71 patients with homozygous mutations or complex heterozygous mutations at the same side of cellular membrane were enrolled.

*P < 0.05, compared with GS patients with a single heterozygous SLC12A3 mutation.

†P < 0.05, compared with GS patients with SLC12A3 mutations intracellularly.

‡P < 0.05, compared with GS patients with SLC12A3 mutations transmembranally.
patients in previous publications and limited the study population to Chinese to avoid the interference of ethnicity. The study is limited for the possible measurement error among different hospitals, although the reference range for serum potassium and magnesium was very similar in different hospitals.

In conclusion, we report a Chinese patient of GS disease with severe clinical manifestations of recurrent tetany. Genetic analysis identifies a novel link between p.R399P mutation in NCCT and GS symptoms, and its homozygous mutation type and mutation site at the extracellular domain of NCCT may correlate with the severe clinical manifestations based on the literature review on the associations between GS manifestations and SLC12A3 mutations in Chinese.

**Authorship**

MX, HB, ZL, and XG: designed the whole study. XG and ZL: diagnosed the patient with GS. MX: collected the information of the patient and her family and contacted the patient for routine follow-up. HL: carried out the kidney biopsy and made pathological diagnosis under light microscopy. HW and ZZ: carried out the electron microscopy examinations. MX and HB: wrote the article and made the literature review.

**Conflict of Interest**

All authors state that they have no conflict of interests.

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Supporting Information
Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Methods of genotype analysis.

Figure S1. Location of p.R399P SLC12A3 mutation at the extracellular long hydrophilic loop of NCCT.