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Clinical and genetic analyses of Chinese patients with Gitelman syndrome

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ABSTRACT. To evaluate the genotype-phenotype relationship of Gitelman syndrome in Chinese patients. We selected patients with Gitelman syndrome presenting hypokalemia. Medical history, clinical manifestations, laboratory test results, and imaging data of these patients were collected for analysis. Target gene sequencing was performed to evaluate the genotype-phenotype relationship. Gitelman syndrome was diagnosed based on medical history, clinical manifestations, laboratory test results, and imaging data. The causative gene for Gitelman syndrome, *SLC12A3*, and the causative gene for the classic Bartter syndrome, *CLCNKB*, were screened for disease-causing mutations by direct sequencing. Clinical diagnoses of ten patients were consistent with Gitelman syndrome. Disease-causing mutations in the *SLC12A3* gene were found in six patients. Among the variants, T60M in exon 1 was the hot spot in Chinese patients. Additionally, we found a small deletion of ACGG in exon 3 and L671P in exon 16; these have not been reported in previous studies. No disease-causing mutations were observed in the other four patients. Since mutations in the *SLC12A3* and *CLCNKB* genes are not present in all patients with

clinical manifestations of Gitelman syndrome, genetic screening after clinical diagnosis is essential.

Key words: Gitelman syndrome; *SLC12A3*; *CLCNKB*; Gene mutation

INTRODUCTION

Gitelman syndrome (GS) is an autosomal recessive renal tubular disease with clinical manifestations similar to those of Bartter syndrome (BS). The main clinical features of this disease are hypokalemia, hypomagnesemia, low urinary calcium, and high aldosterone levels with normal blood pressure. The prevalence of GS in the Caucasian population is approximately 1/40,000. The rate of heterozygous carriers can reach 1%, making it one of the most common hereditary renal tubular diseases (Knoers and Levtchenko, 2008). However, the incidence of GS in the Chinese population remains unreported. Mutations in the *SLC12A3* gene located on the long arm of chromosome 16 may cause GS (Galli-Tsinopoulou et al., 2010). *SLC12A3* encodes the thiazide sensitive sodium chloride cotransporter (NaCl cotransporter or NCCT). More than 140 mutations have been reported in the *SLC12A3* gene (Simon et al., 1996). Mutations in the *CLCNKB* gene encoding chloride channels have also been found in a small number of GS patients.

In the present study, medical histories, clinical manifestations, laboratory test results, and imaging data were collected from patients with GS, and target gene sequencing was performed to evaluate the genotype-phenotype relationship.

MATERIAL AND METHODS

Study subjects

Out of the 50 patients treated for hypokalemia from January 2014 to December 2014 at the Department of Endocrinology of the First Affiliated Hospital of China Medical University, 10 patients were selected for analysis in this study based on the clinical diagnostic criteria of GS. These included six males and four females, aged 18 to 65 years, with a course of the finding or symptoms of hypokalemia of 0.5 to 10 years (Table 1). The Hospital Ethics Committee of the First Hospital of China Medical University approved the study, and all the patients provided written informed consent.

Diagnostic criteria

The clinical diagnosis of GS patients was based on the following criteria: hypokalemia, alkalosis, high urinary potassium (>25 mM/24 h), hypomagnesemia (<0.66 mM), low urinary calcium/creatinine ratio (<0.2), and increased activity of the renin-angiotensin system but normal blood pressure. Patients with metastatic hypokalemia, gastrointestinal loss of potassium, renal tubular acidosis, or medication history including treatment with cascara, diuretics, or ethanol were excluded from this study (Sinha et al., 2012).

Laboratory tests

The blood electrolyte level was assessed with an automatic biochemical analyzer. ACTH and cortisol levels were determined using the chemiluminescence immunoassay method. Plasma renin activity, plasma angiotensin, and plasma aldosterone were measured using a radioimmunoassay.

Genetic testing

Genomic DNA was extracted from peripheral blood using a DNA extraction kit (Tiangen Biotech Beijing Co., Ltd., Beijing). The polymerase chain reaction (PCR) product was purified using an agarose gel extraction kit (Tiangen Biotech Beijing Co., Ltd.). All exons and exon-intron boundaries of the *SLC12A3* and *CLCNKB* genes were amplified and sequenced as described in previous literature (Simon et al., 1996; Yu et al., 2010). When compound heterozygous mutations were suspected, the PCR products were inserted into the pCR2.1 TOPO vector (TA Cloning Kit, Invitrogen) and reamplified from 15 independent positive clones for direct sequencing (Shanghai Simplegen Medical Laboratory and Beijing BGI Sequencing).

In silico functional prediction of mutations

Functional prediction of mutations was performed using the online protein prediction software package PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and PROVEAN/SIFT (http://provean.jcvi.org/).

RESULTS

Clinical data and laboratory test results for the ten patients with GS are shown in Table 1. Among these patients, nine patients had high urinary potassium, and one patient had severe hypokalemia with urinary potassium at the upper limit of normal range. All ten patients had hypomagnesemia, low urinary calcium, and increased activity of renin-angiotensin but normal blood pressure. Clinical diagnosis was made after accounting for metastatic hypokalemia, gastrointestinal loss of potassium, renal tubular acidosis, and history of medication that could induce potassium loss in these patients.

Genetic testing results are shown in Table 2 and Figure 1. Six patients had functional mutations in the *SLC12A3* gene. Among them, one carried a homozygous mutation of T60M, three of them carried compound heterozygous mutations, and two of them carried a single heterozygous mutation. Among the variants, T60M in exon 1 was the hot spot in Chinese patients. In addition, there was a small deletion of ACGG in exon 3 and L671P in exon 16; these have not been reported in previous studies. Disease-causing mutations were not detected in the other four patients. Functional prediction using PolyPhen-2 and PROVEAN/SIFT showed that all these point mutations can impair protein function (Table 3). None of the ten patients carried mutations in the *CLCNKB* gene.

Table 1. Demographic features and laboratory test results of 10 patients with GS.

Patient No.	1	2	3	4	5	6	7	8	9	10	Reference value
Gender	M	M	M	F	M	F	M	M	F	F	-
Age (years)	18	22	44	28	55	65	23	41	24	35	-
Duration (years)	10	2	10	5	9	0.5	2	1	4	8	-
Main symptoms	None	Fatigue	Fatigue, muscle weakness	Fatigue, palpitation	Numbness	Fatigue	Fatigue	Fatigue, numbness	Fatigue, muscle weakness	Numbness	
BMI (kg/m^2)	20.0	21.9	26.9	21.5	29.0	21.1	22.3	23.5	21.4	22.8	-
Blood pressure (mmHg)	120/80	120/70	139/89	93/62	130/80	139/70	110/70	105/75	120/69	115/72	<140/90
Serum pH	7.441	7.417	7.432	7.436	7.431	7.580	7.423	7.431	7.432	7.419	7.35-7.45
Serum potassium (mM)	2.79	2.24	3.03	2.67	2.99	3.03	2.88	3.11	2.65	2.94	3.5-5.3
Serum magnesium (mM)	0.56	0.35	0.88	0.71	0.73	0.47	0.76	0.81	0.54	0.62	0.78-1.28
ACTH (pg/mL)	14.8	19.5	25.0	11.5	8.7	24.0	18.4	19.4	15.9	21.2	7.2-63.3
Cortisol (nM/L)	315.7	347.7	327.7	443.3	433.8	567.0	421.5	328.6	432.3	358.4	171.0-536.0
Aldosterone (ng/mL)	0.08	0.20	0.13	0.07	0.12	0.22	0.11	0.18	0.14	0.09	0.06-0.17
Renin (ng/mL)	5.60	2.40	2.70	3.10	5.51	8.51	4.22	2.31	2.11	1.59	0.05-0.79
Urinary potassium (mM/24 h)	62.06	76.84	29.15	43.94	63.69	35.81	49.38	43.54	55.62	61.39	-
Urinary Calcium (mM/24 h)	0.22	3.52	3.57	0.21	0.20	0.20	2.88	0.22	0.39	0.44	3.74-7.23
Urinary Ca/Cr ratio	0.004	0.034	0.038	0.003	0.004	0.003	0.027	0.003	0.004	0.005	>0.2

BMI = body mass index; ACTH = adrenocorticotropic hormone; Aldo = aldosterone; PRA = plasma renin activity; Cr = creatinine.

Table 2. Screening of *SLC12A3* and *CLCNKB* genes.

Patient	*SLC12A3*	*CLCNKB*
1	rs4784733, rs2304483, rs5803, rs3764264, rs2278490, rs2278489, rs185927948(L849H)*, rs55840684	rs2015352, rs5257, rs1889788, rs71493533
2	rs371443644(T60M)*, rs2304479,	rs2015352, rs5257, rs1889788, rs71493533
3	rs4784733, rs2304479, rs5803	rs2015352, rs5257
4	rs4784733, rs371443644(T60M)*, rs2304479, rs5803, rs2278490, rs55840684, rs753523115(D486N) *	rs2015352, rs5257, rs7367494
5	rs4784733, rs2304478, rs2304483, rs3764264, rs2278490, rs2278489, rs5804, rs55840684	rs2015352, rs5257
6	rs4784733, rs2304479, rs2304483, rs5803, rs3764264, rs2278490, L671P*	rs2015352, rs5257
7	rs4784733, rs13306690, rs2304478, rs13306671, rs2278490, rs12448372, rs56410912, rs13306687, rs3816117, rs3816118, rs2289114	rs5257, rs1889788, rs71493533
8	rs13306689, rs4784733, rs2304479, rs2278489, rs55840684	rs1889788, rs71493533
9	rs13306689, rs4784733, rs5804, rs12448372, rs56410912, rs13306687, rs3816117, rs2289114, rs181865675(N359K) *, c.493-496delACGG*	rs5257
10	rs4784733, rs999662, rs2304478, rs2304479, rs2304480, rs753523115(D486N) *, rs12708965(R928C) *	rs2015352, rs5257, rs1889788, rs71493533

*Pathogenic mutation.

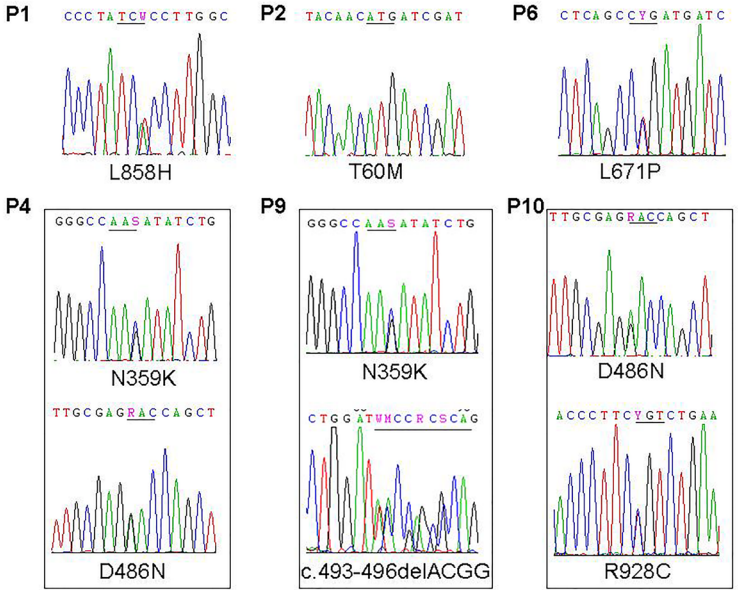

Figure 1. Genetic test results of *SLC12A3* screening. Six patients had functional mutations in the *SLC12A3* gene. Patient #2 carries a homozygous mutation of T60M; patients #1 and #6 carry single heterozygous mutations (L858H and L671P, respectively); and patients #4, #9, and #10 carry compound heterozygous mutations (N359K/D486N, N359K/c.493-496delACGG, and D486N/R928C, respectively).

Table 3. Functional prediction of mutations in the *SLC12A3* gene.

	T60M	N359K	D486N	L849H	R928C	L671P
PolyPhen-2*	Probably damaging (1.0)	Probably damaging (1.0)	Probably damaging (1.0)	Probably damaging (1.0)	Probably damaging (1.0)	Probably damaging (1.0)
SIFT#	Deleterious (-4.98)	Deleterious (-5.71)	Deleterious (-4.75)	Deleterious (-6.14)	Deleterious (-4.58)	Deleterious (-6.68)

*The closer the values come to one, the greater the likelihood of loss of function mutations. #Values equal to or less than -2.5 indicate loss of function mutations.

DISCUSSION

In 1966, Gitelman and coworkers first reported three female patients, aged 22-47 years, with clinical manifestations similar to those of Bartter syndrome as well as hypomagnesemia and low urinary calcium (Yang et al., 2006). The disease was later given the name GS (Lemmink et al., 1998). The development of GS is attributable to mutations in the *SLC12A3* gene located on the long arm of chromosome 16 (Gitelman et al., 1966; Galli-Tsinopoulou et al., 2010). To date, more than 140 *SLC12A3* mutations have been reported in the Human Gene Mutation Database (HGMD), including missense mutations, splice site mutations, nonsense mutations, and frame shift mutations, the majority being missense mutations. Compound heterozygous mutations have been reported in more studies than homozygous mutations. Genetic testing of a group of patients clinically diagnosed with GS shows that T60M may be the most common mutation in the Chinese population (Shao et al., 2007). However, not all patients with GS carry mutations in *SLC12A3*, suggesting that there may be other genetic loci manifesting similar phenotypes (Qin et al., 2009).

In this study, all ten patients had been recently treated in our department for hypokalemia, and presented typical features of GS such as hypokalemia, hypomagnesemia, and renal potassium loss along with normal blood pressure. Since Batter syndrome and Gitelman syndrome have somewhat overlapping phenotypes, we further screened the patients for mutations in the target genes, *SLC12A3* and *CLCNKB*, to ascertain the genetic basis of GS in these patients. We found that six patients carried *SLC12A3* mutations. Patient #2 carried the homozygous T60M mutation, which has been reported to be a common mutation in Chinese and Japanese patients with GS (Maki et al., 2004; Shao et al., 2007). Patient #1 carried the heterozygous L858H mutation, which has been reported exclusively in Japanese patients with GS (Monkawa et al., 2000; Maki et al., 2004; Aoi et al., 2007). We hypothesized that the heterozygous L858H mutation is not sufficient to cause GS, and that there may be variations in gene regulatory regions or unknown mutations in other genes that lead to phenotypes similar to GS. In the present study, we analyzed only the known target genes of classic Batter syndrome and did not find any pathogenic mutations. In addition, studies in Japanese patients have shown that individuals carrying the heterozygous L858H mutation have significantly different serum potassium levels from those who do not (Naraba et al., 2005). It has even been proposed that heterozygous mutations or polymorphisms in the *SLC12A3* gene might significantly affect blood pressure levels (Tago et al., 2004; Liang et al., 2015). In this study, Patient #6 carried the heterozygous L671P mutation, which had not been previously reported. The impact of this mutation on protein function was predicted to be similar to that of other known deleterious mutations; L671P is located in the conserved regions of the protein, impairing its normal function. Our results suggest that the L671P mutation may be a novel gene mutation that can lead to the GS phenotype. The other three patients (#4, 9, and 10) carried compound heterozygous mutations (N359K/D486N, N359K/c.493-496delACGG, and D486N/R928C). Among these mutations, N359K, D486N, and R928C have been described in GS patients (Simon et al., 1996; Lemmink et al., 1998; Qin et al., 2009), whereas the small deletion in exon 3 was novel, to the best of our knowledge. This 4-base pair deletion can cause a frameshift in exon 3, resulting in the predictable termination at amino acid 169 and a lack of the C-terminal of the protein. No abnormalities were found in the *CLCNKB* gene in patients with single heterozygous mutations. The absence of abnormalities in the *CLCNKB* and *SLC12A3* genes in four patients suggests that other factors may contribute to the GS phenotype (Monkawa et al., 2000; Qin et al., 2009).

After treatment with potassium supplement combined with potassium magnesium aspartate, and/or spironolactone, the clinical symptoms of hypokalemia (e.g., fatigue, flaccid

paralysis) in these six patients were relieved. However, hypomagnesemia and hypokalemia could not be entirely corrected, indicating that the ion disorder in GS patients is difficult to treat and requires long-term use of a combination of medications.

GS is a disease with good prognosis and slow progression; only two cases have been reported to develop end-stage uremia (Bonfante et al., 2001; Calò et al., 2003). However, the disease is incurable, and long-term affliction with GS can deteriorate the patients' quality of life and increase their risk of chronic renal insufficiency. Genetic screening can help in early diagnosis and targeted treatment. The current treatment involves the use of a combination of medications including potassium supplement, magnesium supplement, prostaglandin synthetase inhibitors, and aldosterone antagonists. Blood prostaglandin E2 (PGE2) levels tend to be elevated in patients with GS. However, a vast majority of patients with GS have normal PGE2 levels, leading some researchers to believe that treatment with cyclooxygenase inhibitors is ineffective (Kurtz, 1998). The clinical manifestations of ten cases in this study suggest that patients with refractory hypokalemia may be affected by GS, and these patients should undergo genetic diagnostic tests as early as possible to confirm clinical diagnosis, improving their prognosis as well as their quality of life.

Conflicts of interest

The authors declare no conflict of interest.

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