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Original Article

Two Japanese Patients with Gitelman Syndrome

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Abstract. Gitelman syndrome (GS) is a renal tubular disorder characterized by hypokalemia, hypomagnesemia, metabolic alkalosis and hypocalciuria due to defective tubular reabsorption of magnesium and potassium. This disease is caused by mutations of the thiazide-sensitive Na-Cl cotransporter (NCCT) gene, SLC12A3. Manifestations of GS are heterogeneous, from asymptomatic to mild symptoms of cramps and easy fatigue, to tetany and paralysis. Polydipsia, polyuria, and nocturia are also frequent in GS patients. Here we describe two Japanese patients with GS followed as nocturnal enuresis. In the first patient, occasional muscle cramps, easy fatigue and headache led to the diagnosis of GS. The parents of this patient reported that he had been affected by polydipsia and polyuria, especially nocturnal enuresis from early childhood. The second patient was referred to our clinic because of muscular weakness and cramps. He had a past history of transient muscle weakness and muscle cramps. He had also suffered from nocturnal enuresis since 3 yr of age. Laboratory findings of these patients were consistent with those of GS. Sequencing analysis of the SLC12A3 gene from two patients showed four mutations, which were previously reported. In our two patients, their manifestations had been underestimated and the correct diagnosis was delayed. GS is generally likely to be benign, however signs of GS are found in early childhood. Especially, we must recognize that nocturnal enuresis is frequent in symptoms of GS.

Key words: Gitelman syndrome, Na-Cl cotransporter (NCCT), SLC12A3 gene, nocturnal enuresis, polyuria, polydipsia

Introduction

Gitelman syndrome (GS) is characterized by hypokalemic metabolic alkalosis and increased plasma aldosterone level and renin activity in combination with hypomagnesemia and low urinary calcium excretion, caused by defective tubular reabsorption of magnesium and potassium (1, 2). Symptoms of GS patients range

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from asymptomatic, to mild symptoms of cramps and fatigue, to severe manifestations such as tetany and paralysis. Salt craving, polydipsia, polyuria, and nocturnal enuresis are also prominent symptoms (1–4). GS is usually diagnosed in early adulthood during routine laboratory examinations, however there are some reports of children with GS (4–6). The age at presentation is usually 5 yr or more. Cramps in a febrile illness, abdominal discomfort or constipation often lead to medical attention. It is then evident from past history that diffuse muscle pain, muscle weakness, and occasional carpopedal spasms are present. However, manifestations during childhood and adolescence are sometimes underestimated (3, 4).

GS is caused by genetic mutations of SLC12A3, which encodes the thiazide-sensitive Na-Cl cotransporter (NCCT) (7). NCCT is selectively expressed in the distal convoluted tubule of the renal nephron where it represents the main ion cotransporter system. Human SLC12A3 is located on q13 of chromosome 16 and it consists of 26 exons. To date, more than 100 mutations of the SLC12A3 gene have been reported, and these mutations are present throughout the entire gene (5–12). In Japanese patients with GS, several mutations have been identified and one missense mutation (L623P) is particularly frequent, presumably due to a founder effect (5, 9, 11, 12).

Here, we report the two cases of GS patients. Molecular analysis identified four mutations of the SLC12A3 gene. Clinically, it was noted that these two patients had a past history of easy fatigue, occasional muscle pain, weakness, and nocturnal enuresis, however GS had not been suspected.

Case Reports

Patient 1

A 10-yr-old Japanese boy was referred to our hospital because of headache and easy fatigue. His parents were not consanguineous. He had complained of easy fatigue and headache for the past three years. He had also suffered from nocturnal enuresis from early childhood and had been treated with desmopessin acetate (DDAVP) at another clinic. At the time of the earlier treatment his serum levels of potassium and magnesium were not evaluated. On admission, his height was 130 cm (–1.7 SD for normal Japanese boy) and body weight was 26 kg. His blood pressure was 98/40 mmHg. His urine volume was 2000–3000 ml/day. Laboratory findings are shown in the Table 1.

Hypokalemia and hypomagnesemia were found. Increased bicarbonate level and a positive base excess were evident. Plasma aldosterone and plasma renin activity were high. Urinary calcium excretion was low and urinary sodium and chloride excretions were high. There was no nephrocalcinosis. Based on these findings, the boy was diagnosed as having GS. Treatment with potassium (2.3 mmol/kg/day) and magnesium (0.4 mmol/kg/day) supplementation was begun. After this treatment, serum levels of potassium and magnesium remained in the subnormal or lower normal range, however the symptoms of muscle weakness, easy fatigue and polydipsia have improved considerably. Urine volume was reduced to 1000–1500 ml/day. Before treatment nocturnal enuresis was observed four to five times a week, but after treatment the frequency was reduced to once or twice a week.

Patient 2

A 10-yr-old Japanese boy was admitted to our hospital because of periodic paralysis of the limbs and muscular weakness. His parents were not consanguineous. The boy had a past history of occasional muscle cramps during acute febrile illness, polydipsia and nocturnal enuresis from early childhood. His nocturnal enuresis was observed almost every day. His parents reported that he had a food habit of salt craving. His

Table 1 Laboratory findings of Patients 1 and 2

	Normal range	Patient 1		Patient 2	
Blood					
Na (mEq/l)	(136–145)	139		139	
K (mEq/l)	(2.8–4.0)	2.3		2.1	
Cl (mEq/l)	(119–124.5)	93		95	
Ca (mg/dl)	(9.0–10.2)	9.3		9.4	
P (mg/dl)	(3.8–6.1)	4.1		5.1	
Mg (mg/dl)	(1.9–2.3)	1.2		1.4	
Blood gas analysis (vein)					
pH (mmHg)	(7.35–7.45)	7.42		7.43	
HCO_3 (mEq/l)	(21–28)	31.2		30.8	
BE	(–3–3)	9.7		5.4	
Plasma renin activity (ng/ml/h)	(0.2–3.1)	11		17.4	
Plasma aldosterone (ng/dl)	(5–18)	57		31.2	
Urine		before	after	before	after*
Na (mEq/day)	(130–260)	295	341	171	210
K (mEq/day)	(27–40)	38.5	100	55	97
Cl (mEq/day)	(140–260)	257	356	161	256
Mg (mg/day)	(20–130)	192	224	ND	ND
Ca/Cr (mg/mg)	(>0.2)	0.07	0.12	0.01	0.09

ND, not determined. *Data from before and after treatment.

height was 128 cm (–1.6 SD) and weight, 26 kg (–0.7 SD). His blood pressure was 100/60 mmHg. His urine volume was 1500–2500 ml/day. His laboratory findings are presented in the Table 1. Hypokalemia and hypomagnesemia, hyperaldostermonism and hyperreninemia were accompanied by elevated levels of bicarbonate and a positive base excess. The urinary calcium/creatinine ratio was markedly low. Nephrocalcinosis was not found. Thus, a diagnosis of GS was made. Potassium (2 mmol/kg/day) and magnesium (0.4 mmol/kg/day) substitution failed to fully correct hypokalemia and hypomagnesemia. However, the patient's urine volume decreased to approximately 1000 ml/day and the frequency of his nocturnal enuresis is now once a week. The clinical symptoms of muscle pain have disappeared.

Method

This study was approved by the institutional review board, and informed consent for DNA analysis was obtained from both patients' parents. Twenty-six exons of the SLC12A3 gene were amplified by polymerase-chain-reaction (PCR) and these amplified products were subjected to direct sequencing by specific primers according to previous reports (5, 8). Amplitaq-Gold (Perkin-Elmer, CA, USA) and its standard buffer were used in all reactions. All exons were amplified by PCR under the following conditions: initial denaturation at 95°C for 7 min, followed by 30 cycles at 94°C for 1 min, 62–65°C for 1 min, and 72°C for 1 min. The amplified products were electrophoresed, purified by low melting agarose gel and subsequently sequenced according to the manufacturer's protocol on an automated sequencer (Applied Biosystems Model

373A DNA sequencer, CA, USA).

Results

Two mutations were identified in the patient 1. One heterozygous mutation was L623P in exon 15 (Fig. 1A); the other mutation was a heterozygous two T base deletion of nucleotides 2543 and 2544 in exon 21(2543-2544del TT) (Fig. 1B). Patient 2 had two heterozygous missense mutations. One was G439S, and the other was S555L (Fig. 2B). DNA samples from the parents of these two patients were not available.

Discussion

In our two patients, we identified four mutations previously reported in the SLC12A3 gene. Patient 1 had L623P and 2543-2544delTT mutations. Both mutations have been previously reported in Japanese patients with GS (5, 9, 11, 12). The 2543-2544delTT mutation leads to a premature stop codon in exon 21, thus eliminating the C-terminus of NCCT protein. In the second patient, G493S and S555L mutations were found. Both mutations have also been reported in GS patients (3, 8). As DNA samples from the parents of these two patients were not available, we could not determine whether they were compound heterozygotes.

As mentioned above, patients with GS are usually diagnosed in early adulthood. However, there are some reports of children with GS. Schmidt *et al.* (6) have reported 4 children with GS. Carpopedal spasms during acute febrile illness lead to the diagnosis. However, Cruz *et al.* (3) have described that symptoms of GS, especially fatigue and difficulty to perform are often underestimated. Although our patients had past histories of muscle weakness and easy fatigue from early childhood, they had not sought medical attention. The second patient had a food habit of salt craving. It has been reported that salt craving is observed in approximately 70% of GS patients (3). Accordingly, we need to have a better understanding of the variable symptoms that are common to GS to aid diagnosis of this disease.

Another important point of our patients' cases was nocturnal enuresis. Polydipsia, polyuria and nocturia are prominent symptoms of GS. It has been reported that GS patients rate polyuria and nocturia as particularly problematic (3). Our two patients had suffered from nocturnal enuresis and one patient was treated with DDAVP. Nocturnal enuresis is common in pediatric practice; thus, the differential diagnosis of nocturnal enuresis should include GS.

In conclusion, we have reported the cases of two patients with GS. Variable clinical manifestations, which should lead to suspicion of GS, are already present in early childhood. Early diagnosis would enable us to start treatment.

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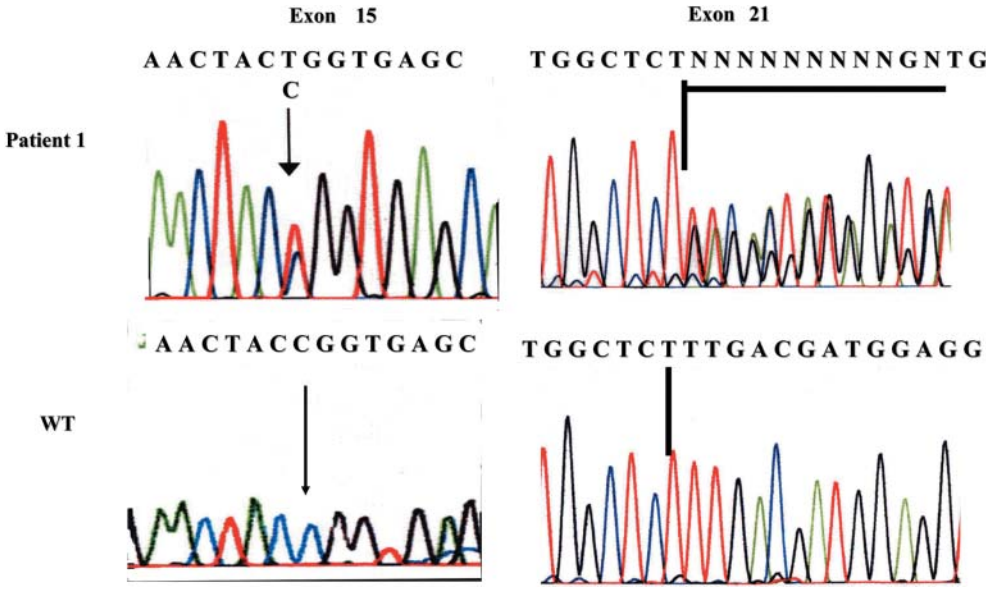

Fig. 1 Sequencing analysis of Patient 1. (A) An arrow indicates the L623P mutation of exon 15. Double peaks of C and T were present in Patient 1(arrows). (B) A heterozygous two base (TT) deletion (nucleotide 2543 and 2544) was found. Note that after the deletion site, double peaks were shown (underline).

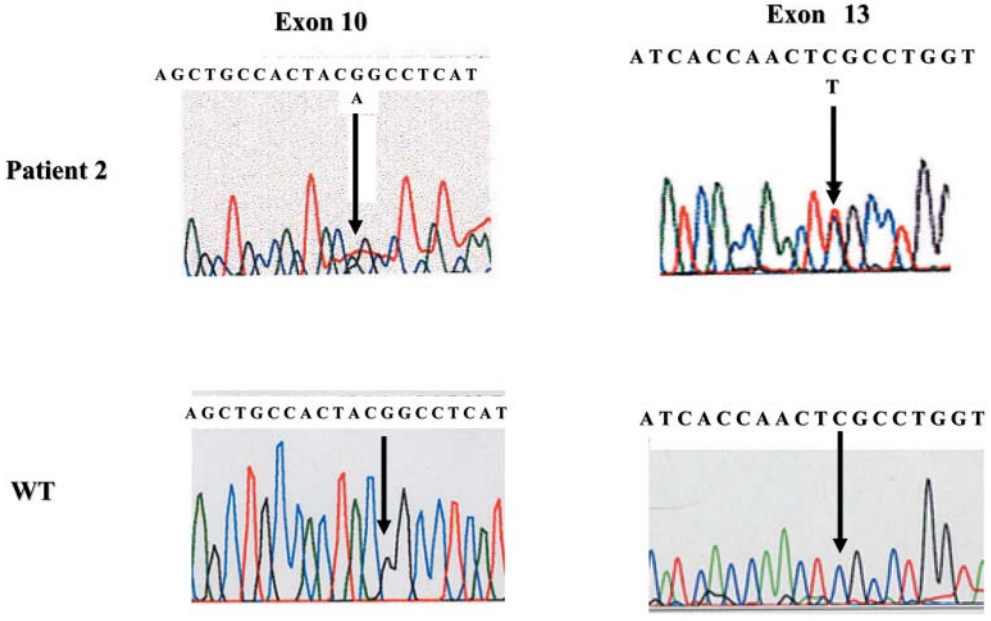

Fig. 2 Sequencing analysis of Patient 2. (A) G439S in exon 10. In Patient 2, double peaks of G and A were present (arrows). (B) S555L was identified in exon 13. C and T nucleotides (arrows) were present in the sequence of Patient 2.

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