A Novel Compound Heterozygous Mutation of Gitelman’s Syndrome in Japan, as Diagnosed by an Extraordinary Response of the Fractional Excretion Rate of Chloride in the Trichlormethiazide Loading Test

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Abstract

Gitelman’s syndrome (GS), an inherited disorder due to loss of function of ion channels and transporters such as Na-Cl co-transporter (NCCT) in distal convoluted tubules, is characterized by hypokalemia, hypomagnesemia, hypocalciuria, metabolic alkalosis and hyperreninemic-hyperaldosteronism. A 39-year-old man was admitted to our hospital because of muscle weakness with such intractable disorders. We performed a thiazide-loading test, which revealed a poor response of the fractional excretion rate of chloride compared to healthy subjects. Based on these data, the clinical diagnosis of GS was made. Gene-sequencing analysis revealed compound heterozygous mutations of c.539C > A and c.1844C > T in SLC12A3, which is newly reported in Japanese GS.

Key words: hypokalemia, hypomagnesemia, hypocalciuria, Bartter syndrome, Gitelman’s syndrome

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Introduction

Mild to moderate hypokalemia is a common finding in the clinical setting. However severe hypokalemia presenting limb paresis is fairly rare. The pathogenesis of hypokalemia in the adult is often due to potassium (K) loss from the gastrointestinal duct or kidney but is rarely due to inherited disorders. Hypokalemic salt-losing tubulopathies have been reported to constitute a distinct set of inherited renal disorder (1). The Bartter like syndrome has been known to have several defective ion transporters or channels in the tubules which are the sodium-chloride co-transporter (NCCT) in the distal convoluted tubule, the sodium-potassium-chloride co-transporter (NKCC2) or the renal outer medullary potassium channel in the loop of Henle and the chloride channels ClC-Ka and ClC-Kb or their beta-subunit Barttin in the combined distal convoluted tubule and loop of Henle. Furthermore, gene mutations related to the defective ion transporters or channels have also been defined. In clinical practice, the information and understanding about the pathophysiology can be, or can only be obtained from clinical presentation and non-invasive procedures. Here we show a case of a middle-aged man that presented with a first episode of severe muscle weakness due to severe hypokalemia which turned out to be an inherited disease of renal potassium loss.

Case Report

A 39-year-old Japanese man, with a history of stable schizophrenia, came to our emergency department because of muscle weakness and difficulty in walking for several days. Two months before admission, he began having watery stool a few times a day without nausea or vomiting. He
Denied taking any supplements or any other over-the-counter-drugs. He drank several liters of water, a moderate amount of alcohol and a glass of vegetable juice almost every day as a habitual way to relax. In the previous three months, his alcohol consumption had increased because of stress during work. He had a 15-year history of stable schizophrenia for which he had been treated with risperidone and promethazine hydrochloride and had no recent changes in prescription. He had no other previous medical problems.

He was 169.9 cm tall and weighed 75.8 kg. His vital signs were as follows: blood pressure 113/64 mmHg, heart rate 106 bpm, body temperature 36.6°C, respiratory rate 16 per minute and SpO₂ 94% in room air. On physical examination, he was alert and appeared to be euvolemic with wet oral mucosa, slightly wet axilla, normal capillary refill time (i.e. <2 seconds) and no change in body weight. His thyroid gland was not swollen and no nodules were palpable. Heart sound was regular with no murmurs and breath sounds were normal. Abdominal examination was unremarkable. Upper and lower extremities showed muscle strength of 3/5 with no laterality and no abnormal reflexes.

As shown in the Table 1, routine laboratory workup showed hypokalemia (1.8 mEq/L), hypomagnesemia (1.5 mg/dL) with QT elongation on electrocardiogram, hypophosphatemia (1.4 mg/dL) and elevation of serum creatinine kinase. He was diagnosed as hypokalemic paralysis with hypokalemic myopathy, and was hospitalized. He was slowly given potassium chloride (KCl) intravenously by drip infusion throughout the night. We followed up hypophosphatemia without medications, because there was no sign of respiratory depression, which is the most deleterious complication with hypophosphatemia.

On day 2, as shown in Table 1, serum K dropped from 1.8 to 1.6 mEq/L, and trans-tubular K gradient (TTKG) turned out to be inadequately elevated to 8.7. Moreover, initial arterial blood gas analysis showed combined acid-base disorders such as metabolic alkalosis (pH 7.513, HCO₃ 26.8 mmol/L, corrected HCO₃⁻ = 30.0) with high urine-chloride and metabolic acidosis with elevated anion gap (anion gap 16.2). Elevated anion-gap proved to be a result of hyperlactatemia (24.5 mg/dL) which was probably caused by daily alcohol intake. Such metabolic acidosis with elevated anion gap was spontaneously corrected during the period of hospitalization but the metabolic alkalosis sustained with resistive hypokalemia and hypomagnesemia. Tubular maximum for phosphate corrected for GFR (TmP/GFR) was shown to be slightly low (2.1) but hypophosphatemia improved to 2.1 mg/dL at that time without phosphate supplementation. Thyroid function tests showed normal values (thyroid stimulating hormone 1.75 μU/mL, free T₄ 1.48 ng/dL). Therefore, thyrotoxic periodic paralysis was ruled out.

At this point, he was diagnosed as hypokalemic myopathy and mild rhabdomyolysis due to hypokalemia induced by chronic alcohol intake and exacerbated by diarrhea. While other multiple electrolyte disorders such as hypomagnesemia and hypophosphatemia with an elevated excretion rate seemed to be consistent with alcohol abuse, the pathogenesis of enhanced TTKG was still to be elucidated. Then we ordered plasma renin activity and plasma aldosterone concentration at bed rest in the morning of day 2.

We kept supplying more than 80 to 200 mEq/day of KCl by drip infusion and/or as tablets for a few days, but when we tried to reduce potassium supplementation, hypokalemia slowly recurred still showing high TTKG (Fig. 1). Hypomagnesemia was also resistant to orally supplied magnesium (Mg) without causative diarrhea. Only hypophosphatemia was spontaneously resolved without medications, probably because of improved nutrition status.

Several days after, results of plasma renin activity (PRA) and plasma aldosterone (PA) on day 2 were turned out to be 34.1 ng/mL/hr and 189 pg/mL, respectively. Reevaluation at day 15, after replenishment of extracellular fluid, still showed high PRA (PRA, 25.4 ng/mL/hr PA, 230 pg/mL) which lead us to conclude that inadequately elevated TTKG was due to hyperreninemic hyperaldosteronism.

We supposed that hyperreninemic hyperaldosteronism with resistive hypokalemia, metabolic alkalosis and lack of hypertension indicated the possibility of renal tubular diseases like Bartter’s syndrome and Gitelman’s syndrome (GS). High fractional excretion rate of Mg with moderately low serum Mg concentration and hypocalciuria were more compatible to the latter. To further investigate the pathophysiology, we underwent a thiazide loading test as reported.
by Colussi et al. (2). Instead of using 50 mg of hydrochlorothiazide, we used 10 mg of trichlormethiazide which is much easily available in Japan. Four healthy volunteers underwent the same test as a control. Informed consent was obtained from all subjects. The results revealed that compared to the control group, the patient had an apparently weak response ($\Delta$ FECl%) to trichlormethiazide (Fig. 2). As a result, we concluded that NCCT of the patient had severely reduced function and clinically diagnosed him as GS.

On day 29 of hospitalization when the patient was discharged, hypokalemia and hypomagnesemia were corrected to 3.7 mEq/L with 72 mEq/day of KCL tablets and to 1.6 mEq/L with 37.5 mEq/day of Mg oxide tablets, respectively. On day 39, we started spironolactone 25 mg/day, because hypokalemia progressed to 2.9 mEq/L. On day 49, serum potassium rose to 4.1 mEq/L, but diarrhea recurred, so we reduced the dose of Mg oxide to 250 mg/day and eventually stopped Mg oxide at the next visit because of lasting diarrhea and little therapeutic effect in resolving hypomagnesemia. Serum potassium concentration was almost 2.8-3.5 mEq/L with 48 mEq/day of KCl tablets and no symptoms occurred during that period.

With the consent of the patient and his mother and approval of the institutional ethics committee, we performed gene sequence analysis of the NCCT for both. We detected heterozygous mutations of c.539C > A (T180K, p.Thr180Lys) and c.1844C > T (S615 L, p.Ser615Leu) in the patient and heterozygous mutation of S615 L in his mother (Fig. 3).

**Discussion**

This Asian man in the late 30s presented to the emergency room with subacute muscle weakness due to severe hypokalemia. At first, from the history of two-month watery stool and increase in habitual alcohol consumption in this patient, we thought of an acquired illness rather than an inherited disease. Psychiatric medications generally do not directly cause hypokalemia although together with hypokalemia they may cause an elongated QT interval (3). Thyrotoxic periodic paralysis, which has been reported to affect Asian men frequently (4), was ruled out by blood test in this case. Further tests revealed that in spite of hypokalemic and euvolemic state, plasma renin activity and aldosterone levels were rather high. It seemed to cause an inappropriately high urinary excretion of potassium. Hypomagnesemia

Figure 1. Course of hypokalemia and hypomagnesemia with therapy. Upper graph shows the amount of supplementation given. Hypokalemia recurred after titrating KCl supplementation to 48 mEq/day.

Figure 2. Result of trichlormethiazide loading and maximal increase in fractional chloride clearance ($\Delta$FECl%) of the patient and controls.
and hypophosphatemia are often observed in alcohol abusers due to increased urinary losses. The former is proposed to be induced by suppressed excretion of parathyroid hormone and/or steroid hormones, and the latter is advocated to be functionally deteriorated by direct alcohol toxicity to proximal tubular cells (5). Therefore, hypophosphatemia tends to improve faster with abstinence for a few days rather than other electrolyte disorders.

Gitelman’s syndrome was first introduced with characteristics of hypokalemia, metabolic alkalosis and normal blood pressure under hyperreninemic hyperaldosteronism coexisting with hypocalciuria and hypomagnesemia (6). It has been reported that patients do not present with symptoms before school age and are usually diagnosed in late childhood or in adult life. Later Bettinelli et al. (7) described the following clinical criteria: 1) hypomagnesemia (<0.65 mmol/L, which is almost 1.6 mg/dL) in the presence of inappropriately high magnesium excretion (fractional excretion of magnesium >4.0%); 2) hypokalemia (<3.6 mmol/L) in the presence of inappropriately high potassium excretion (fractional excretion of potassium >16.0%); and 3) urinary calcium/creatinine molar ratio <0.10 (which is almost 35 mg/gCr). Although FEK on admission had been lower than the thresholds of this criteria, FEK rose to 16.4% when serum K was still 3.4 mEq/L at day 17, and to 31% when serum K was 2.9 mEq/L at day 39 (Fig. 1). Similarly, FEMg rose to 6.4-10.3% when serum Mg was still 1.3-1.6 mg/dL. The transient low FEK and FEMg on admission were probably caused by severe deficits of these electrolytes. It has been reported that a practical bedside test of thiazide administration (2) demonstrates that thiazides in these patients do not show much response. We used trichlormethiazide, the most commonly used thiazide in Japan, and followed the protocols by Colussi et al. (2), which were effective in distinguishing GS with Bartter’s syndrome or pseudo-Bartter.

We performed gene analysis tests to confirm our clinical diagnosis and support the results obtained by trichlormethiazide loading test. Inactivating mutations in the SLC12A3 gene that encodes the NCCT at the distal convoluted tubule were identified as the cause for GS (8). Homozygous T180K mutation has been reported to be a fairly common mutation in Japanese GS (9). Interestingly, heterozygous S615L mutation in a case of GS in one study was identified with no other mutation of coding sequence of NCCT (2) while all of the other cases of GS with S615L mutation were reported in compound heterozygotes (2, 10). With genomic DNA direct sequencing technique targeted for only SLC12A3 and CLC-Kb locus of the GS patient in that study, possible mutations, for example, in the promoter region or intron of SLC12A3 or CLC-Kb may affect the phenotype interdependently with S615L mutation of SLC12A3.

The patient’s mother denied any symptoms such as polydipsia, polyuria and muscle weakness, and was never pointed out of any problems in obligated health checks at the local community. No problems were indicated during pregnancy. The patient’s father also denied of any medical problems. Based on this information, it is likely that heterozygous T180K or S615L mutation interdependently causes GS. One report of GS lacking hypomagnesemia with heterozygous mutation in S615L suggests that T180K impairs magnesium reabsorption (11).

As far as we know, this is the first case of a compound heterozygous mutation involving T180K (maternal allele) and S615L (probably paternal allele) on the NCCT to present as GS. There are some reports suggesting that the frequency of GS genotype is higher than the estimated incident rate of GS (9, 12). It may mean that GS genotypes do not always cause a typical GS phenotype and that genetic and/or epigenetic factors are related to functions of the channel (12).

In conclusion, we encountered a case of hypokalemic paralysis, which was diagnosed as GS both clinically and genetically. Genetic analysis revealed a unique compound heterozygous T180K and S615L on the NCCT gene. Trichlormethiazide loading test may be very helpful to confirm the diagnosis of GS simply at the bedside and to sort out possible candidates for genetic analysis.

The authors state that they have no Conflict of Interest (COI).

References


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