Case Report

A Case of Gitelman's Syndrome with Decreased Angiotensin II–Forming Activity

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Gitelman's syndrome (GS) is a variant of Bartter's syndrome (BS) characterized by hypokalemic alkalosis, hypomagnesemia, hypocalciuria and secondary aldosteronism without hypertension. A 31-year old Japanese man who had suffered from mild hypokalemia for 10 years was admitted to our hospital. He had metabolic alkalosis, hypokalemia and hypocalciuria. Since he had two missense mutations (R261C and L623P) in the thiazide-sensitive Na-Cl cotransporter (TSC) gene (SLC12A3), he was diagnosed as having GS. He showed hyperreninism and a high angiotensin I (Ang I) level, whereas his angiotensin II (Ang II) and aldosterone levels were not elevated. His angiotensin converting enzyme (ACE) activities were normal, and administration of captopril inhibited the production of Ang II and aldosterone. We evaluated the Ang II–forming activity (AIIFA) of other enzymes in his lymphocytes. Interestingly, chymase-dependent AIIFA was not detected in the lymphocytes. Together, these results suggest that the lack of chymase activity resulted in the manifestation of GS without hyperaldosteronism. (*Hypertens Res* 2006; 29: 545–549)

***Key Words*: Gitelman's syndrome, angiotensin II, aldosterone, chymase**

Introduction

In 1966, Gitelman *et al*. reported three adult patients with intermittent episodes of muscle weakness and tetany, hypokalemia, and hypomagnesemia, but no history of polyuria or growth retardation (*1*). These three patients suffered from what was later called Gitelman's syndrome (GS), also known as the hypocalciuric variant of Barter syndrome (BS), a disease characterized by hypokalemia, hypomagnesemia, and hypocalciuria (*2*). This disorder was suggested to be due to a defect in electrolyte transport at the distal convoluted tubules that was sensitive to thiazide diuretics (*3*, *4*). The gene (SLC12A3) encoding the thiazide-sensitive Na-Cl cotransporter (TSC) was found to be responsible for GS, and so far several TSC gene mutations have been reported (*5–10*). Moreover, GS is characterized by sodium wasting, low blood pressure, and secondary hyperaldosteronism. Here, we report a case of GS with hyperreninism and high angiotensin I (Ang I) but without elevated angiotensin II (Ang II) or hyperaldosteronism.

Methods

TSC Gene Analysis

To detect the genetic mutation, direct sequencing analysis was conducted for all 26 exons of the TSC gene. According to the guidelines of the institutional ethical committee, the purpose and the detailed procedure of the genetic analysis were

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Table 1. Laboratory Examination of This Patient in 1992 and 2003

	Year		Normal range
	1992	2003	
Serum K (mmol/l)	2.9	3.0	
Blood pressure (mmHg)	124/60	134/74	
Urinary K (mmol/day)	26.1	43.9	
PRA (ng/ml/h)	15.0	12.0	0.3–2.9
Plasma Ang I (pmol/l)	490	401	<85
Plasma ACE activity (IU/l/37°C)	8.8	7.8	8.3–21.4
Plasma Ang II (pmol/l)	13.4	19.1	<21
PAC (nmol/l)	0.5	0.55	0.08–0.44

PRA, plasm renin activity; Ang I, angiotensin I; ACE, angiotensin converting enzyme; Ang II, angiotensin II; PAC, plasma aldosterone concentration.

Table 2. Results of Renin Stimulating Test

	Before	After
Standing test		
PRA (ng/ml/h)	9.8	19
Ang I (pmol/l)	587	926
Ang II (pmol/l)	21	80
PAC (nmol/l)	0.58	0.66
Captopril test		
PRA (ng/ml/h)	6.9	80
Ang I (pmol/l)	293	>1,930
Ang II (pmol/l)	40	14
PAC (nmol/l)	0.39	0.26
BP (mmHg)	117/54	110/60

PRA, plasma renin activity; Ang I, angiotensin I; ACE, angiotensin converting enzyme; Ang II, angiotensin II; PAC, plasma aldosterone concentration; BP, blood pressure.

explained and informed consent was obtained from the patient.

Laboratory Measurements

Plasma renin activity, and plasma Ang I, Ang II and aldosterone concentrations were measured by radioimmunoassay.

Ang II–Forming Activities in Lymphocytes

White Blood Cell Isolation

Peripheral blood was drawn from vein. Mononuclear cells (L) containing mainly lymphocytes, or polymorphonuclear cells (P) containing mainly neutrophils, eosinophils and basophils were prepared using Lymphoprep or Polymorphoprep solution (both from Nycomed Pharma AS, Oslo, Norway), respectively (*11*, *12*). Leukocyte fractions of the isolated L and P samples were determined by Giemsa staining. The L fraction was composed of 77.5% lymphocytes, 20.6% monocytes and 1.3% polymorphonuclear cells, whereas the P fraction was composed of 99% polymorphonuclear cells.

Preparation of Homogenate Fractions

The pellets were resuspended in 50 mmol/l NaH_2PO_4 buffer, pH 7.4 (containing 100 mmol/l NaCl and 10 mmol/l $MgCl_2$), and homogenized with a glass/glass homogenizer on ice. The protein concentration of the fraction homogenate was measured by BCA Protein Assay Reagent (Pierce, Rockford, USA).

Assessment of Ang II Formation

Angiotensin II–forming activity (AIIFA) from Ang I (Peptide Institute, Inc., Osaka, Japan) was determined according to a previously described method with some modification (*13*). Cells prepared as described above were incubated with Ang I (0.2 mol/l) at 37°C for 30 min. The amount of Ang II formed was analyzed by high-performance liquid chromatography (HPLC) using a C_{18} reverse-phase column (2.2 × 25 cm; Vydac, Hesperia, USA) with a 15-min linear acetonitrile gradient (4–16%) in 25 mmol/l triethylamine-phosphate buffer, pH 3, at a flow rate of 2 ml/min. AIIFA levels were expressed as nmol or pmol of Ang II formed per min per mg of protein. Captopril (1 mmol/l)- or chymostatin (0.1 mmol/l)-inhibitable (both from Sigma Chemical Co., St. Louis, USA) and aprotinin (0.24 mmol/l) (Bayer, Osaka, Japan)-insensitive Ang II formations were expressed as ACE or chymase-dependent AIIFA, and the resultant captopril- or chymostatin-insensitive and aprotinin-inhibitable activity was presented as cathepsin G–dependent AIIFA.

Case Report

A 31-year-old Japanese male was admitted to our hospital with an approximately 10-year history of hypokalemia. His hypokalemia (K 2.9 mmol/l) had first been detected at 20 years of age, and he was admitted to a hospital at that time. Upon examination at that hospital, he was suspected of having BS. Moreover, he had hyperreninism with a high plasma concentration of Ang I, but without hyperaldosteronism or

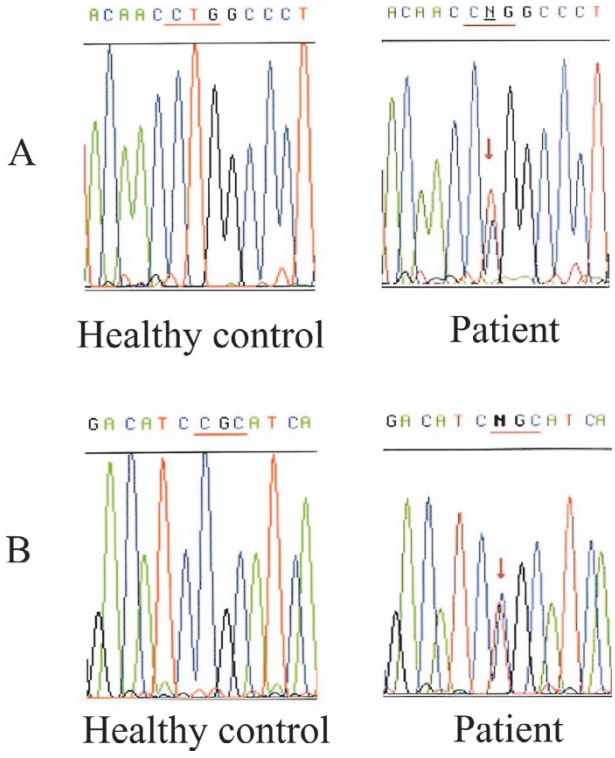

Fig. 1. *Results of the TSC gene analysis. A: DNA sequence analysis in exon 15 of TSC. The patient has a heterozygous transition (T→C) in exon 15, resulting in a Leu (CTG) -to-Pro (CCG) substitution at residue 623. B: DNA sequence analysis in exon 6 of TSC. The patient has a heterozygous transition (C→T) in exon 6, resulting in an Arg (CGC) -to-Cys (TGC) substitution at residue 261.*

high Ang II levels. Because his mild hypokalemia was persistent and proteinuria developed, he was referred to our hospital 11 years later, in 2003. Upon admission to our hospital, his blood pressure was 134/74 mmHg. His growth was normal (body weight, 62.2 kg; height, 171 cm). He did not have muscle weakness, paralysis or edema. He had no history of chronic diarrhea or vomiting and no history of diuretic use. His parents were not consanguineous. His serum electrolyte concentrations were as follows: sodium: 139 mmol/l; potassium: 3.0 mmol/l; chloride: 94 mmol/l; calcium: 2.48 mmol/l; phosphorus: 1.16 mmol/l; and magnesium: 0.74 mmol/l. He also showed metabolic alkalosis (pH 7.481, HCO_3^- 33.8 mmol/l). His urinary sodium and potassium excretions were high (sodium: 133 mmol/day; potassium: 43.9 mmol/day), while his urinary calcium excretion was extremely low (0.34 mmol/day *vs*. the averaged value of our outpatients: 4.4±2.2 mmol/day, n=1,327). His calculated fractional excretion (FE_{Ca}) value was 0.09%. Based on these findings, a diagnosis of GS was made. An endocrinological examination revealed hyperreninism associated with a high concentration of plasma Ang I, while the Ang II and aldosterone concentrations were normal (Table 1). Ang II and aldosterone were not stimulated by standing (Table 2). On the other hand, captopril significantly inhibited the production of Ang II and aldosterone.

Table 3. Ang II–Forming Activities (pmol/min/mg Protein) in Lymphocytes

	Healthy men (same age)	Present case
ACE	41.9	8,870.6
Chymase	20.9	0
Cathepsin G	1,439.3	77,437.7

ACE, angiotensin converting enzyme; Ang II, angiotensin II.

By using polymerase chain reaction (PCR)–amplification and direct sequencing, two mutations were detected in the TSC gene (Fig. 1). One C-to-T base substitution was identified at nucleic acid position 1868 in the Na-Cl cotransporter cDNA, which lies in exon 15 of the TSC gene. The mutation causes an amino acid substitution of proline for leucine at amino acid position 623 (L623P). A C-to-T base change was also found at nucleic acid position 781, which causes an amino acid substitution of cysteine for arginine at amino acid position 261 (R261C). These mutations were detected in a compound heterozygous form. The former has already been reported in Japan (*5*), while the latter has not been reported.

Table 3 shows the activities of Ang II generating enzymes (ACE, chymase and cathepsin G) in the lymphocytes. In this patient, chymase-dependent AIIFA was not detected.

Percutaneous renal biopsy revealed mild mesangial proliferation. On the immunofluorescence microscopy, no immunoglobulin deposition in the mesangial area was noted. Based on these findings, a diagnosis of mesangial proliferative glomerulonephritis (non IgA) was made. Mild juxtaglomerular cell hyperplasia was also observed.

Discussion

Diagnosis of GS

In this case, hypokalemia and hypomagnesemia were mild, while hypocalciuria was remarkable. In addition to these clinical findings, a previously reported missense mutation in TSC (L623P) was detected. Among 1,852 subjects recruited from the Suita study, Tago *et al*. detected the T180K, A569V, L623P, R642C, and L849H heterozygote genotypes in 56, 14, 1, 1, and 47 subjects (*14*). He concluded that the overall frequency of GS mutations was 0.0321. However, almost all of the reported GS mutations were case reports without functional confirmation. Naraba *et al*. assessed the functionality of the two most prevalent mutations in Japanese, T180K and L849H, using a mammalian cell expression system (*15*). The L849H mutation was confirmed to be a loss-of-function mutation and appears to be responsible for GS. The functional significance of the L623P and R261C mutations on GS have

not been confirmed.

Renin-Angiotensin-Aldosterone System

GS usually presents with secondary hyperaldosteronism due to salt wasting. In this patient, hyperreninism with a high concentration of Ang I (two to five fold greater than normal) was observed, but hyperaldosteronism and high Ang II concentration were absent. These symptoms persisted for 10 years. In another Japanese case report of GS (serum potassium: 2.6 mmol/l), plasma Ang I and Ang II concentrations were extremely elevated (11,737 pmol/l and 228 pmol/l, respectively) but hyperaldosteronism was mild (0.66 nmol/l) (*16*). Gibbs *et al*. showed that plasma aldosterone may not be as high as expected for the degree of hyperreninemia in BS and GS because of the low total body potassium (*17*). Hypokalemia may also explain the relatively mild hyperaldosteronism in GS. Since high Ang I concentration was not associated with high Ang II concentration in our case, we speculated that an impairment of the Ang II–generating system may have been responsible for the lack of hyperaldosteronism. Serum ACE activity was normal. Administration of captopril suppressed plasma Ang II and plasma aldosteron concentration (PAC), and led to marked elevation of plasma renin activity (PRA) and plasma Ang I concentration, suggesting that ACE effectively induced the generation of Ang II in this patient. We suspect that, in the present case, GS may have primarily impaired the activity of other Ang II–generating enzymes (*i.e.*, chymase or cathepsin G).

Ang II–Generating Enzymes

In addition to ACE, there are other enzymes, including chymase or cathepsin G, with the ability to generate Ang II *in vivo* (*18*). Chymase has high specificity for Ang I and its Ang II–generating ability is higher than that of ACE *in vitro* (*19*). We examined the activities of these three Ang II–generating enzymes (ACE, chymase and cathepsin G) in lymphocytes of the present case. Chymase-dependent AIIFA was not detected and the other enzyme activities were extremely high. Thus, we speculated that a decrease in the activity of chymase was responsible for the absence of hyperaldosteronism in the present case. Moreover, the lack of hyperaldosteronism led to mild hypokalemia in this case. It is not clear that the absence of chymase-dependent AIIFA in lymphocytes can be extrapolated to other tissues. For this reason, it will be important to determine the activity and expression of chymase in the renal tissue of this patient in a future work. An analysis of the chymase gene would also be useful. Ono *et al*. sequenced the human mast cell chymase gene (CMA1) and found 13 single-nucleotide polymorphisms, two of which were loss-of-function mutations (*20*). Since we have not sequenced the chymase gene, we are unable to exclude the possibility that the nonsense mutation of the chymase gene resulted in the lack of chymase activity in this patient. Since the activities of ACE and cathepsin G were extremely elevated, it is not clear whether a suppression of chymase activities could explain the lack of elevated Ang II and aldosterone. In 1981, Bergstein *et al*. reported three patients with a disease that resembled BS; these patients had low plasma concentrations of aldosterone despite their elevated renin activity (*21*). Umeki *et al*. also reported a patient who had hyperreninism associated with Ang I elevation but who lacked Ang II elevation or hyperaldosteronism (*22*). He concluded that the decreased affinity of ACE to the substrate Ang I (so-called ACE dysfunction syndrome) was the cause of this phenomenon. We speculate that chymase-dependent AIIFA were impaired in these cases. There are no reports that show the activity of Ang II–generating enzymes in GS. The severity of clinical findings is variable in BS or GS. Chymase activity in BS or GS may be responsible for the clinically severe forms of these diseases. Moreover, the novel mutation of TSC (R261C) found in this patient may have played a role in his mild hypokalemia and hypomagnesemia.

GS with Glomerulonephritis

Our patient had histologically diagnosed mesangial proliferative glomerulonephritis. To our knowledge, GS associated with glomerulonephritis has not been reported. Because his renal function is preserved and his hypokalemia and proteinuria are mild, we are currently treating him by recommending potassium rich foods.

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