Bartter syndrome representing digenic-based salt-losing tubulopathies presumably accelerated by renal insufficiency

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5 Word count of the manuscript: 2584

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ABSTRACT

Bartter syndrome (BS) and Gitelman syndrome (GS) are autosomal recessive disorders usually caused by homozygous or compound heterozygous mutations in causative genes. In some patients, these two syndromes cannot be discriminated based on clinical features or mutation type; thus, a single disease concept, salt-losing tubulopathies (SLTs), has been used instead. Despite the existence of several SLT causative genes, cases of digenic heterozygous mutations in two different genes are extremely rare. Here, we report the case of a 36-year-old woman with renal insufficiency and hypokalemia caused by an SLT. To evaluate the SLT phenotype, we performed next generation sequencing (NGS) with a gene panel including *SLC12A3*, *SLC12A1*, *CLCNKB*, and *CLCNKA* as well as laboratory examinations and diuretic loading tests. The results of the diuretic loading tests were consistent with a GS phenotype, while the NGS results showed that the patient had heterozygous mutations in *SLC12A1* and *CLCNKB*. Both genes have been associated with BS, suggesting that the SLT was caused by digenic heterozygous mutations in two different genes. To date, only a few SLT cases caused by digenic heterozygous mutations in two different genes have been reported. The digenic SLT phenotype in the patient was presumably accelerated by moderate renal insufficiency.

Keywords: *SLC12A1*, *CLCNKB*, Bartter syndrome, Salt-losing tubulopathies

INTRODUCTION

Bartter syndrome (BS) and Gitelman syndrome (GS) are autosomal recessive renal tubular salt-wasting disorders characterized by hypokalemia and metabolic alkalosis. BS usually develops in neonates or during childhood and has relatively severe symptoms, whereas GS is diagnosed in childhood and adulthood and has relatively mild symptoms. Type 3 BS often presents clinical features similar to GS and thus is difficult to distinguish from GS. Although the prevalence of hypomagnesemia and hypocalciuria is higher in GS than in BS, it is difficult to completely discriminate these two pathophysiologies; therefore, BS and GS have recently been regarded as a single disease: hereditary salt-losing tubulopathies (SLTs) [1,2].

The SLT phenotype may vary depending on patient background; for example, a previous report [3] described a patient with an *SLC12A3* heterozygous mutation who developed GS with hypokalemia following the identification of anti-SS-A antibodies, despite the fact that the *SLC12A3* heterozygous mutation alone does not cause hypokalemia in patients with GS [4,5]. The hereditary form of SLT is autosomal recessive and is usually caused by homozygous or heterozygous mutations in the same gene; however, here, we report a patient with an SLT caused by digenic heterozygous mutations in two different BS-causative genes, with no mutations in other causative genes.

CASE REPORT

Clinical and Genetic Analysis

A 36-year-old woman was admitted to our hospital with renal dysfunction and hyperuricemia. As her symptoms were initially thought to be caused by dehydration, intravenous rehydration was performed. Following discharge from the hospital, she was followed up as an outpatient; however, her general fatigue and renal dysfunction did not improve, and the hyperuricemia worsened. Moreover, her blood examination showed hypokalemia 2 months after the initial admission. She was readmitted to our hospital for further investigation 8 months after the initial admission. There were no remarkable features in her personal, familial, or social life history. In addition, she had not exhibited renal dysfunction or serum potassium disorder until one year prior to her initial admission. At the second admission, her height was 150.8 cm, weight was 41.7 kg, and blood pressure was 102/66 mm Hg. No rales or murmurs were heard. There were no noteworthy findings on her chest radiograph image and abdominal computed tomography did not show any kidney calcification. The laboratory findings showed renal dysfunction; serum creatinine, 1.44 mg/dL and estimated glomerular filtration rate (eGFR), 34.4 mL/min/1.73 m^2. Despite the lower serum potassium level (3.1 mEq/L), the urinary potassium level was 47.1 mEq/L, fractional excretion of potassium was 16.8%, and the trans-tubular potassium gradient was 6.6, indicating that the hypokalemia was caused by renal potassium wasting. An arterial blood gas exam showed metabolic alkalosis; pH, 7.47 and HCO_3^-, 28 mEq/L. The plasma renin activity increased as high as 27.3 ng/mL/h and the

plasma aldosterone concentration also increased up to 848 pg/mL. The laboratory data at the second admission are detailed in Table 1. Extreme hyperuricemia was observed; uric acid (UA; 17.2 mg/dL) and UA clearance were thought to be reduced. Diuretic drug loading tests with furosemide and thiazide were conducted to distinguish between BS and GS [6]. The Δ fractional excretion of chloride (FECl) at maximum diuresis (FECl at maximum diuresis - FECl before administration of the diuretic) in the furosemide loading test was 16.75%, indicating an increased response to the administration of furosemide. In contrast, the ΔFECl in the thiazide loading test was 0.72%, indicating a poor response.

Based on these findings, the patient was clinically diagnosed with SLT, conceivably due to GS. We next conducted a comprehensive genetic diagnosis by next generation sequencing (NGS) [7] including *SLC12A3*, a major causative gene of GS; *SLC12A1*, a major causative gene of BS; and *KCNJ1*, *CLCNKB*, *BSND*, *CLCNKA*, *HNF1B*, and *CASR*. In addition, we conducted copy number variation (CNV) analysis using the NGS data. Notably, while no mutation was found in *SLC12A3*, a heterozygous nonsense mutation (c. C1411T: p.R471X) was found in *SLC12A1* (NM_000338), which was previously reported as a disease causative mutation of type 1 BS [8]. Similarly, a heterozygous splice region mutation (c.1845 + 1G> A), not previously reported, was identified in *CLCNKB* (NM_000085), another causative gene of type 3 BS. No causal mutations were identified in *ABCG2*, *SARS2*, *UMOD*, *HPRT1*, *G6PC*, or *MUC1*, which are the genes responsible for

hereditary hyperuricemia, including the CNV results. Oral potassium chloride was initiated; the dose was increased from 8 to 16 mEq/day, resulting in an improved serum potassium level. In addition, administration of febuxostat led to attenuation of the hyperuricemia. eGFR decline and serum potassium level transition showed similar trends throughout the clinical course, as shown in Fig. 1.

DISCUSSION

BS and GS are characterized by hypokalemia, metabolic alkalosis, hyper renin, hyperaldosteronemia, and autosomal recessive congenital tubular dysfunction. Clinically, BS can be classified as a severe neonatal type that develops during the neonatal period or a relatively mild classical type that is discovered in early childhood. BS generally shows normal serum magnesium levels and hypercalciuria and is characterized by a decreased urinary chloride (Cl^-) response in the furosemide loading test. GS can be distinguished from BS based on hypomagnesemia, hypocalciuria, mild clinical symptoms, and a decreased urinary Cl^- response in the thiazide loading test. However, a previous report has demonstrated BS variance; for instance, only 45.6% of BS patients diagnosed in adulthood exhibited both hypomagnesemia and hypocalciuria and 16.7% of type 3 BS patients showed both hypomagnesemia and hypocalciuria [9]. Based on recent advances in molecular biology, BS and GS have been classified as type 1 to type 4b (type 5) BS and GS; the

conventional clinical classifications of neonatal type BS, classical type BS, and GS do not necessarily correspond to the clinical symptoms associated with their causative gene mutations. Therefore, BS and GS have been regarded as a single disease concept and comprehensively termed SLTs [1,2].

As this patient exhibited adult onset and an increased response to the furosemide loading test and a decreased response to the thiazide loading test were observed, the patient was clinically thought to have GS and a genetic examination was performed to detect mutations in GS associated genes. While no mutation was identified in *SLC12A3* (NCC), a major GS causative gene, a heterozygous nonsense mutation previously reported by Urbanová et al. [8] was detected in *SLC12A1* (NKCC2), a causative gene of type 1 BS. In addition, a novel, previously unreported splice mutation (c.1845 + 1G> A) was identified in *CLCNKB*, a causative gene of type 3 BS. Interestingly, this is a rare mutation that has not been registered with large allele frequency databases such as ExAC [10] or 1000 Genomes [11]. Although it is difficult to evaluate splice site mutations using an in silico pathological significance prediction score, the combined annotation dependent depletion score (CADD) [12], which partially covers splice sites, was as high as 23.2 (> 15) and the genomic evolutionary rate profiling score, which measures the evolutionary conservation of a particular genetic sequence across species, was highly conserved at 4.63. Therefore, this mutation appears to have pathological significance via gene transcription abnormalities. BS is an autosomal recessive

inheritance disease and is thought to develop if a homozygous mutation or compound heterozygous mutations exist within the same gene; however, this case differed from ordinary BS cases, as the etiology involved digenic heterozygous gene mutations in two different BS-causative genes.

Previous reports have shown that gene mutations in *CLCNKB* and *CLCNKA*, which code for two basolateral Cl^- channels in the thick ascending loop of Henle (TAL), cause SLTs via a defect in Barttin [13,14]. Generally, SLC12A1 is located in the apical membrane, while CLCNKB is located in the basolateral membrane of the TAL; however, these two channels are also expressed in the distal tubule [9,15]. Although the mechanism by which digenic heterozygous gene mutations cause SLTs remains unknown, there have been five previous reports of different ion channel proteins, located on different sides of the TAL, that are associated with SLTs [16–18]. We hypothesize that the patient in this report had moderate renal insufficiency and that the nonsense mutation in *SLC12A1* might have accelerated the digenic-based SLT phenotype. Moreover, the results of the diuretic test suggested damage in the distal tubules, which may reflect the expression of SLC12A1 and CLCNKB1 isoforms in the distal tubules and may be associated with hypocalciuria [9,15]. In addition, renal insufficiency may have also played a role in the SLT phenotype in this case and may have affected the results of the diuretic test. However, a previous study reported that the effects of thiazides, including natriuresis and the lowering of blood pressure, are maintained even in patients with stage 4 chronic kidney disease [19]. The eGFR and serum potassium levels exhibited similar

tendencies, suggesting that renal function played an important role in SLT development in this case (Fig. 1). The genetic background and clinical features of previous case reports and this patient are detailed in Table 2.

BS usually develops during the neonatal period and presents severe symptoms; however, this patient exhibited adult onset, suggesting that some acquired factors may affect BS onset in addition to genetic predisposition. Further studies and additional cases are necessary to elucidate whether the existence of digenic mutations in the *SLC12A1* and *CLCNKB* genes plays an important role in SLT development.

This report has some limitations: as our results mainly relied on NGS, deep intronic variants, intergenic variants, or large genomic rearrangements in known pathogenic genes may have been missed. In addition, the existence of mutations in unknown genes not included in the panel can also not be excluded. Moreover, *CLCNKB* and *CLCNKA* share gene sequence homology (94% identical); although the NGS program should be able to distinguish between the genes, it is possible that the algorithm is limited and does not allow for differentiation.

In conclusion, as BS and GS are difficult to clinically distinguish, the concept of SLTs has been used instead recently. The responsible gene does not always correspond to patient phenotype; thus, genetic testing is crucial for a definite diagnosis. In this case, digenic heterozygous mutations in *SLC12A1* and *CLCNKB* are thought to be associated with SLT development. As NGS enabled us to

confirm the gene mutations in this case, the performance of genetic testing should be considered in SLT patients.

ACKNOWLEDGMENTS

Not applicable.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors have declared that no conflict of interest exists.

Research involving Human Participants and/or Animals

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethical Committee of Isahaya General Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Written informed consent for genetic testing and publishing this report was obtained from the patient in accordance with the guidelines of the Ethical Committee of Isahaya General Hospital.

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Table 1. Laboratory data at second admission

Blood cell count			**Biological chemistry**			**Urinary test**		
WBC	5980	/μL	TP	7.9	g/dL	proteinuria	±	
Seg	61	%	Alb	4.6	g/dL	uric blood	–	
Lymph	32	%	AST	20	IU/L	RBC	0 – 1	/HPF
Mono	5	%	ALT	13	IU/L	WBC	0 – 1	/HPF
Eosino	2	%	UA	17.2	mg/dL	U-TP/Cr	0.08	g/gCr
Baso	0	%	BUN	42	mg/dL	NAG	9.6	U/L
RBC	385×10^4	/μL	Cr	1.44	mg/dL	U-Na	40	mEq/L
Hb	11.6	g/dL	Na	138	mEq/L	U-K	60.6	mEq/L
PLT	15.5×10^4	/μL	K	3.1	mEq/L	U-Cl	78	mEq/L
Immunology and hormones			Cl	100	mEq/L	U-Ca	2.0	mg/dL
IgG	1430	mg/dL	Ca	10.0	mg/dL	U-Cr	136.3	mg/dL
IgA	299	mg/dL	P	2.8	mg/dL	U-UA	71.0	mg/dL
IgM	133	mg/dL	Mg	2.1	mg/dL	U-Na	57	mEq/day
C3	93.0	mg/dL	CRP	< 0.05	mg/dL	U-K	33	mEq/day
C4	26.2	mg/dL				U-UA	0.40	mg/kg/h
ANA	< 40		pH	7.47		UACL	2.42	mL/min
renin activity	27.3	ng/mL/h	PCO_2	39	mmHg	UACL/CrCL	4.23	%
Aldosterone	848	pg/mL	PO_2	99	mmHg	U-UA/U-Cr	0.46	
			HCO_3^-	28	mEq/L			
			BE	5.3	mEq/L			

WBC: white blood cell, Seg: segmented neutrophils, Lymph: lymphocyte, Mono: monocyte, Eosino: eosinophil, Baso: basophil, RBC: red blood cell, Hb: hemoglobin, PLT: platelet, Ig: immunoglobulin, C3: complement 3, C4: complement 4, ANA: anti-nuclear antibody, TP: total protein, Alb: albumin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, UA: uric acid, BUN: blood urea nitrogen, Cr: creatinine, Na: sodium, K: potassium, Cl: chloride, Ca: calcium, P: phosphate, Mg:

magnesium, CRP: C reactive protein, BE: base excess, HPF: high power field, NAG: N-acetyl-β-D-glucosaminidase, U-: urinary, UACL: uric acid clearance, CrCL: creatinine clearance.

Table 2. Previous reports of digenic salt-losing tubulopathy mutations and Cl^- channels located in the contralateral sides of the thick ascending loop

Patient	Age (years)	mutated gene	Mutation	$[K^+]$	$[Mg^{2+}]$	urinary Ca excretion	Year	Reference
1-1	6	*SLC12A3*	2534delT	2.5	0.8	NA	2005	Bettinelli et al. [16]
		CLCNKB	compound heterozygous A61D/V149E					
1-2	19	*SLC12A3*	2534delT	↓	[b]	NA	2005	Bettinelli et al. [16]
		CLCNKB	compound heterozygous A61D/V149E					
2-1	19	*SLC12A3*	c.2660+1delG	3.0	0.58	urinary Ca/Cr 0.07	2016	Lee et al. [17]
		CLCNKB	c.1589C > T, p.P530L					
2-2	25	*SLC12A3*	c.539C > A, p.T180K	3.1	0.63	urinary Ca/Cr 0.26	2016	Lee et al. [17]
		CLCNKB	Homozygous c.1830G > A, p.W610[a]					
3	8	*SLC12A3*	p.N359K	3.3	0.49	urinary Ca 0.01 mM/L	2019	Kong et al. [18]
		CLCNKB	p.L94I					
This case	36	*SLC12A1*	c. C1411T: p.R471X	3.1	2.1	urinary Ca 0.5 mM/L		
		CLCNKB	c.1845 + 1G> A					

[a]Represents a termination mutation; [b]in more than half of the determinations.

NA: not available, $[K^+]$: serum potassium concentration (mM/L), $[Mg^{2+}]$: serum magnesium concentration (mM/L), Ca: calcium.

Figure caption

Fig. 1 Clinical course

Figure 1 : Clinical Course