



Combination of furosemide and fludrocortisone as a loading test for diagnosis of distal renal tubular acidosis in a pediatric case

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Abstract

Renal tubular acidosis (RTA) is a rare disease caused by a defect of urinary acidification. The ammonium chloride loading test is the gold standard method for determining the type of RTA. However, because this test has some side effects (e.g., nausea, vomiting, and stomach discomfort), applying this test for pediatric cases is difficult. Recently, a loading test with the combination of furosemide and fludrocortisone was reported to be an alternative to the ammonium chloride loading test, with 100% sensitivity and specificity in adult's cases. We report the first pediatric case of distal RTA in a patient who was successfully diagnosed by a drug loading test with the combination of furosemide and fludrocortisone without any side effects. We also performed genetic analysis and detected a known pathogenic variant in the *SLC4A1* gene. The combination loading test of furosemide and fludrocortisone is a useful and safe diagnostic tool for pediatric cases of RTA.

Keywords Furosemide · Fludrocortisone · Loading test · SLC4A1 · Autosomal dominant distal renal tubular acidosis · Rickets · Short stature

Introduction

Renal tubular acidosis (RTA) is a group of disorders with urinary acidification disability and it causes metabolic acidosis with a normal plasma anion gap without diarrhea. RTA is classified into four types. Type 1 is distal RTA (dRTA), which is caused by impaired excretion of H⁺. Type 2 is proximal RTA caused by reabsorption failure of HCO₃⁻. Type 3 is a mixed type of types 1 and 2. Type 4 is hyperkalemic RTA caused by a lack or failure of aldosterone function at the distal tubule. The ammonium chloride loading test is the gold standard method for determining the type of RTA, especially for the differential diagnosis of types 1 and 2 RTA. Using this test, patients in whom urinary pH remains above 5.5, despite strong acidemia with a pH of 7.3 or less, are diagnosed with dRTA. However, ammonium

chloride stimulates the stomach. Therefore, this loading test often causes abdominal discomfort, nausea, or vomiting. Therefore, the ammonium chloride loading test is not often applied to cases of suspected RTA, especially for children. Therefore, the furosemide loading test was developed as an alternative to ammonium chloride loading with fewer side effects. When the cut off of this test is set to a urinary pH greater than 5.5 for the diagnosis of dRTA, the sensitivity is 100%, but it has a relatively low specificity of 82–89% [1, 2]. A previous report showed that even healthy volunteers had a urine pH less than 5.5 following a loop diuretic of only 50% with oral administration and 75% after intravenous administration. Guerra-Hernandez NE et al. also reported that the urinary acidification test with furosemide alone was performed with 30 children and showed some false positives [3, 4]. These data suggest that the furosemide loading test still has a problem of specificity.

Recent reports showed that a loading test with the combination of furosemide and fludrocortisone could be an alternative to the ammonium chloride loading test with 100% sensitivity and specificity [5, 6]. The authors of these reports regarded a urinary pH greater than 5.3 as the diagnostic criterion for dRTA. However, there have been no reports on this combination loading test applied to children.

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We report here a genetically proven case of autosomal dominant dRTA in a patient with a short stature and rickets in childhood. To the best of our knowledge, this is the first pediatric case report of a loading test by furosemide and fludrocortisone.

Case report

A 4-year-old boy was referred to our hospital because of a short stature and gait abnormality. His parents noticed his gait abnormality and visited a local orthopedic clinic, but a lower limb X-ray examination showed no remarkable findings. He was born at full term, by normal vaginal delivery, with a birth weight of 2780 g, and showed normal development. There was no history of short stature in his family. He often played outside and had no food allergy or unbalanced diet. At the initial visit, his height was 91.9 cm (-2.7 SD) and his weight was 11.8 kg (-2.1 SD) (Fig. 1). He walked in a bow-legged manner. A blood

examination showed hypokalemia, hyperphosphatasemia, hypophosphatemia, and a low 25(OH) vitamin D level. Blood gas analysis showed hyperchloremic metabolic acidosis with a normal plasma anion gap. Urinalysis showed a relatively high urine pH and hypercalciuria (Table 1). X-ray examinations showed cupping and fraying signs on his wrist and knee (Fig. 2). Abdominal ultrasonography showed nephrocalcinosis (Fig. 3). Diuretic tests with furosemide alone and the combination of furosemide and fludrocortisone were conducted following the protocols previously reported [7]. Furosemide was given at a dose of 1 mg/kg by intravenous route and fludrocortisone was given at a dose of 1 mg/1.73 m² by oral. Urine samples were collected every hour from spontaneous voiding for a period of 4 h. The patient was not fasting before tests and allowed drinking water during tests. We left 24 h between tests. And, both showed urine acidification defects (Fig. 4). Although this case showed typical finding for distal RTA, we conducted this test under the consent of the parents to make a definitive diagnosis and

Fig. 1 Growth curve. The black points represent height and body weight at an examination. The arrow shows the point of starting oral alkali therapy

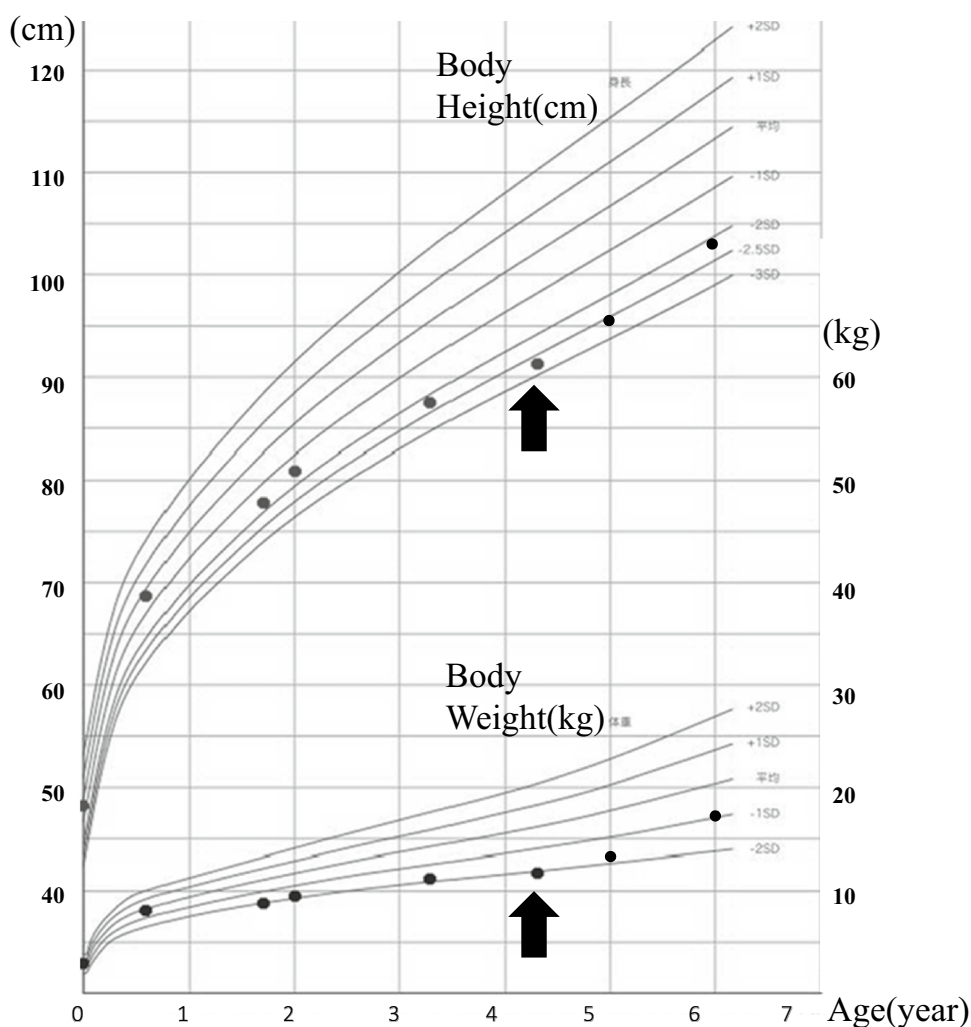
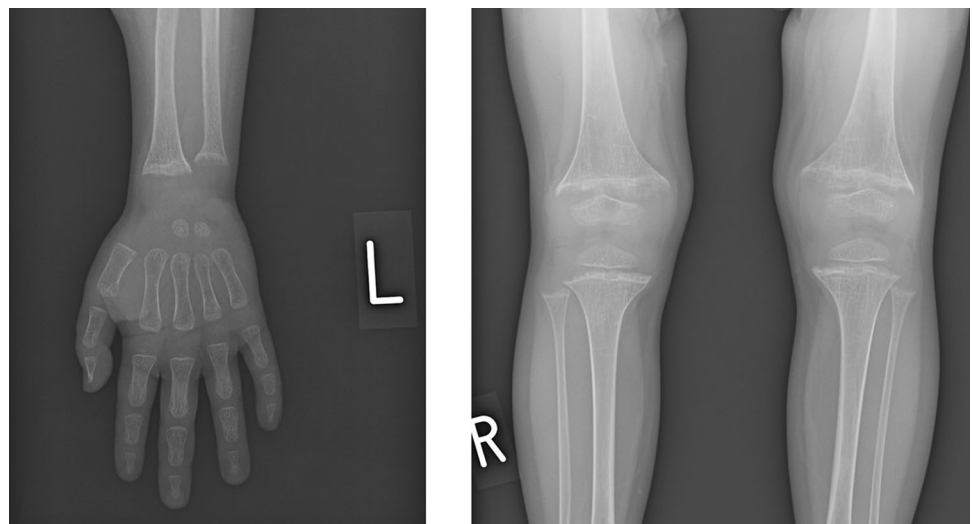


Table 1 Laboratory data of the patient on admission

Blood		Urine	
Venous blood gas		Urinalysis	
pH	7.287	pH	7.5
pCO ₂	31.1 mmHg	Specific gravity	1.01
HCO ₃ ⁻	14.5 mmol/L	Protein	(1+)
Base excess	-10.7 mmol/L	Glucose	(-)
Complete blood count		Blood	(-)
White blood cell	9300/μL	Urine chemistry	
Hemoglobin	16.2 g/dL	Sodium	61 mmol/L
Platelet count	35 × 10 ⁴ /μL	Potassium	25.1 mmol/L
Serum chemistry		Chloride	48 mmol/L
Albumin	4.9 g/dL	Anion gap	38.1 mmol/L
Aspartate amino transferase	26 U/L	Calcium	9.3 mg/dL
Alanine transaminase	9 U/L	Creatinine	28.27 mg/dL
Alkaline phosphatase	2378 U/L		
Blood urea nitrogen	8.1 mg/dL		
Creatinine	0.24 mg/dL		
Sodium	137 mmol/L		
Potassium	2.5 mmol/L		
Chloride	113 mmol/L		
Anion gap	9.5 mmol/L		
Calcium	8.6 mg/dL		
Phosphorus	2.4 mg/dL		
Ammonia	161 μg/dL		

Plasma anion gap: sodium - (chloride + HCO₃⁻), uretic anion gap: sodium + potassium - chloride

Fig. 2 An X-ray at the first examination shows cupping and fraying signs on the patient's wrist and knee



to understand the pathophysiology of this case having severe acidosis. We conducted targeted sequence analysis with a next generation sequencer and detected a known pathogenic missense mutation at exon 14 (c.1766G>A, p.Arg589His) in *SLC4A1* (NM_000342.3) [8] (a list of genes is shown in Table 2). This variant was confirmed by

the Sanger method. We also performed genetic analysis for the parents and neither of them had the same variant. We diagnosed the patient as a sporadic case of autosomal dominant dRTA. After starting oral alkali therapy, the metabolic acidosis and hypokalemia were soon normalized, and his growth rate improved (Fig. 1).

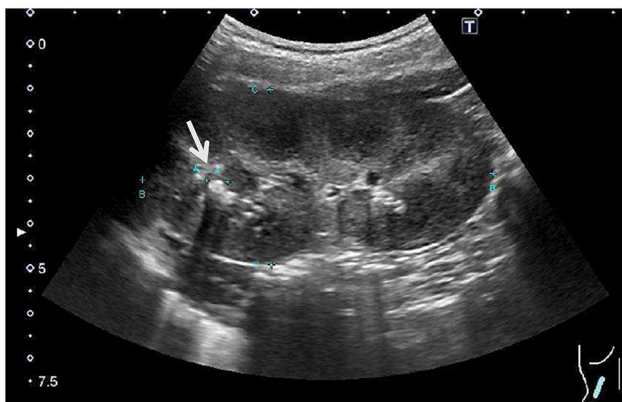


Fig. 3 Abdominal ultrasonography at the first examination shows nephrocalcinosis (arrow)

Discussion

Autosomal dominant dRTA is a rare genetic disease caused by pathogenic variants in the *SLC4A1* gene encoding the chloride–bicarbonate anion exchanger 1. Autosomal recessive dRTA is caused by pathogenic variants in either the *ATP6V1B1* or *ATP6V0A4* gene encoding subunits of the vacuolar H^+ -ATPase. A defect of urinary acidification in the distal nephron leads to hyperchloremic metabolic acidosis with a normal plasma anion gap, and this is frequently accompanied by hypokalemia, hypercalciuria, and nephrocalcinosis. Affected patients usually

show mild phenotypes and present with clinical symptoms in adolescence or adulthood [9–12]. Alonso-Varela et al. reported that the median age at diagnosis of patients with *SLC4A1* gene mutations was 10 years old and they showed relatively mild symptoms [13].

Furosemide loading blocks NKCC2, which is a sodium transporter at the thick ascending limb of Henle’s loop, and a lot of sodium flows into the distal renal tubule and collecting ducts. Furosemide loading enhances reabsorption of sodium from these segments and results in a negative charge of the urine. This condition leads to excretion of proton ions and urinary acidification occurs. Addition of fludrocortisone loading enhances sodium reabsorption by expressing sodium channels in the cell membrane in collecting duct epithelial cells, and also has a direct action to enhance the activity of H^+ -ATPase [5]. Therefore, a combination of furosemide and fludrocortisone loading can enhance urine acidification. However, patients with dRTA lack the ability of excreting proton ions into urine and lack acidification with furosemide loading. In our case, the results of the furosemide loading test and furosemide and fludrocortisone loading test showed a defect of urine acidification. Genetic analysis also detected a known pathogenic variant. We diagnosed our patient as dRTA clinically and genetically.

This study has some limitations, because we conducted this test for only one case and have not conducted any tests on healthy pediatric cases. Recent studies suggested that in incomplete dRTA cases, furosemide and fludrocortisone tests could not replace the ammonium chloride loading test [14–16]. Therefore, careful interpretation of the results is

Fig. 4 Diuretic tests with furosemide alone (a) and the combination of furosemide and fludrocortisone (b) were conducted, and both showed urine acidification defects

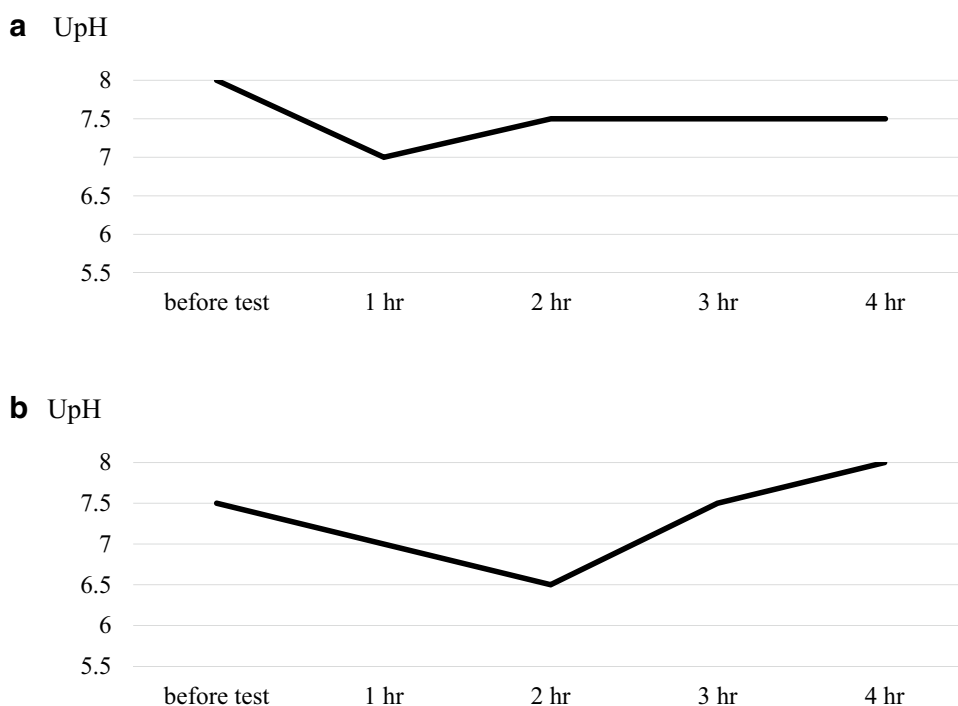


Table 2 Panel used for gene analysis

SLC12A1	CFH	CUL3	COQ2	EMP2
KCNJ1	CFI	WNK4	COQ6	FAT1
CLCNKB	MCP (CD46)	COL4A3	ITGA3	KANK1
BSND	C3	COL4A4	ITGB4	KANK2
CLCNKA	CFB	COL4A5	GLEPP1 (PTPRO)	KANK4
SLC12A3	DGKE	GLA	MYO1E	PDSS2
CASR	THBD	UMOD	ARHGDI1A	PTPRO
MAGED2	ADAMTS13	MUC1	ADCK4	WDR73
CFTR	FN1	SEC61A1	TTC21B	XPO5
CLCN5	SLC4A1	REN	NUP93	ACTN4
OCRL	ATP6V0A4	PKD1	NUP107	ANLN
SLC26A3	ATP6V1B1	PKD2	NUP205	ARHGAP24
KCNJ10	SLC4A4	GANAB	CRB2	INF2
CLDN16	CA2	EYA1	CUBN	LMX1B
CLDN19	EHHADH	SIX2	EMP2	MYH9
FXYD2	SLC34A1	CD2AP	FAT1	PAX2
EGF	CTNS	NPHS1	KANK1	TRPC6
TRPM6	NR3C2	NPHS2	KANK2	WT1
KCNA1	SCNN1A	PLCE1 (NPHS3)	KANK4	WDR73
CNNM2	SCNN1B	SMARCAL1	PDSS2	MAGI2
HNF1B	SCNN1G	LAMB2	PTPRO	
SLC41A3	KLHL3	SCARB2	CUBN	

We detected a pathogenic mutation in the *SLC4A1* gene

Bold values show the responsible genes for renal tubular acidosis

needed with diagnose these cases. Accessing gene diagnostic technology, including next generation sequencing, is becoming easier. However, we believe that the loading test is still a useful tool with high diagnostic value for patients with dRTA, and it can be conducted safely and easily in our facility.

Conclusion

We diagnosed dRTA with a short stature and rickets by diuretic tests using furosemide and furosemide plus fludrocortisone. The combination of furosemide and fludrocortisone as a loading test without side effects in pediatric cases may be useful for diagnosing children with dRTA. A loading test with a combination of furosemide and fludrocortisone is an alternative to the ammonium chloride loading test for pediatric cases. However, more cases need to be collected and analyzed to validate this alternative.

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Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from participant's parents included in the study.

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