

Original Paper

Genotype–Phenotype Analysis in Pediatric Patients with Distal Renal Tubular Acidosis

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Key Words

Distal renal tubular acidosis • Mutations • Sensorineural hearing loss • Nephrocalcinosis • Chronic kidney disease • Growth retardation

Abstract

Background/Aims: Primary distal renal tubular acidosis (dRTA) in children is a rare genetic disorder, and three causative mutated genes have been identified: *SLC4A1*, *ATP6V1B1*, and *ATP6V0A4*. We analyzed the prevalence and phenotypic differences of genetic mutations in children with dRTA. **Methods:** A total of 17 children with dRTA were enrolled in the study. All patients underwent genetic testing for all three candidate genes. **Results:** Pathogenic mutations, including six novel mutations, were detected in 15 (88.2%) patients: dominant *SLC4A1* mutations in ten (58.8%) patients, recessive *ATP6V0A4* mutations in three (17.6%) patients, and recessive *ATP6V1B1* mutations in two (11.8%) patients. Compared to other patients, patients with *SLC4A1* mutations showed an older age of onset (3.7 ± 2.6 years) and less severe metabolic acidosis at initial presentation. All patients developed nephrocalcinosis, and sensorineural hearing loss was observed in two patients with *ATP6V1B1* mutations. Three (17.6%) patients had decreased renal function (chronic kidney disease stage 2), and five (29.4%) patients had persistent growth retardation at the last follow-up. Long-term prognosis showed no genotype–phenotype correlation. **Conclusions:** *SLC4A1* is the most common defective gene in Korean children with dRTA. Patients with *SLC4A1* mutations show later onset and milder disease severity. Long-term follow-up of hearing ability, renal function, and growth is necessary for patients with dRTA.

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Introduction

Distal renal tubular acidosis (dRTA), also referred to as type 1 RTA, is clinically characterized by persistent hyperchloremic metabolic acidosis with normal serum anion gap, hypokalemia, hypercalciuria, and nephrocalcinosis. Metabolic acidosis in this disease is caused by an inability of the α -intercalated cells in the collecting duct to secrete H^+ and to properly acidify urine [1, 2]. In children, dRTA is almost always observed as a primary entity with underlying genetic causes, while in adults, dRTA frequently develops secondarily as a consequence of other diseases including hypergammaglobulinemia, some autoimmune disorders, chronic liver diseases, and chronic renal allograft rejection [2]. To date, at least three genes are known to cause primary dRTA: *ATP6V1B1*, *ATP6V0A4*, and *SLC4A1* [1, 3]. *ATP6V1B1* and *ATP6V0A4* encode the B1 and A4 subunits of the apical H^+ -ATPase pump in α -intercalated cells, respectively, and mutations in these genes cause autosomal recessive (AR) forms of dRTA with or without sensorineural hearing loss (SNHL) [4, 5]. *SLC4A1* encodes the basolateral anion (Cl^-/HCO_3^-) exchanger 1 (AE1) [6, 2]. The majority of *SLC4A1* mutations have been reported in association with the autosomal dominant (AD) form of dRTA [6]. However, AR dRTA with or without accompanying hemolytic anemia caused by *SLC4A1* mutations has also been reported, predominantly from tropical Southeast Asia and other tropical regions [7, 8].

Children with primary dRTA typically present with growth failure, polyuria, hypercalciuria, medullary nephrocalcinosis, nephrolithiasis, and hypokalemia. The progression of nephrocalcinosis may lead to the development of chronic kidney disease (CKD). Therapeutic correction of the metabolic acidosis by adequate alkali supplementation, if started early in life, may enable catch-up growth, arrest of nephrocalcinosis, and preserve renal function [2, 9].

Several genotype–phenotype correlation studies in patients with primary dRTA have been reported, although the results from these studies vary [10–15].

In this study, we analyzed the genotype–phenotype correlation in 17 Korean pediatric patients with dRTA and compared the results with those reported by other studies.

Materials and Methods

Study population

A total of 17 pediatric patients who were admitted to or referred for genetic testing to Seoul National University Children's Hospital with a clinical diagnosis of dRTA from 2002 to 2015 were included in this study. All patients were Korean and none of them were from a consanguineous background. The clinical diagnosis of dRTA was based on laboratory testing of hyperchloremic metabolic acidosis with a normal plasma anion gap, normal renal function, and inappropriately high urinary pH on repeated sampling. None of the patients had so called "incomplete dRTA", which refers to patients with no overt metabolic acidosis at baseline but with urinary acidifying defect (urinary pH >5.3) following the administration of an acid load. Medical records and biochemical data were retrospectively reviewed. The estimated glomerular filtration rate (eGFR) was measured using the bedside Schwartz formula [16].

Mutational screening

Mutational screening was performed for all patients. Genomic DNA was extracted from nucleated cells in the peripheral blood using a commercially available kit (QIAamp DNA Blood Mini Kit; QIAGEN, Hilden, Germany), and all coding exons with flanking introns of *SLC4A1*, *ATP6V1B1*, and *ATP6V0A4* were amplified using PCR followed by direct sequencing. In addition, we also screened *CA2*, which encodes the cytosolic carbonic anhydrase II and causes combined distal and proximal RTA with osteopetrosis [17].

Statistical analyses

Qualitative data were described as frequencies whereas quantitative data were reported as the means \pm standard deviations. The Chi-square test or Fisher's exact test was used, as appropriate, to compare proportions between groups. Quantitative variables were compared using the Mann-Whitney *U* test. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). A *P* value < 0.05 was considered statistically significant.

Ethical Approval

This study was conducted in accordance with the 1964 Declaration of Helsinki and was approved by the Seoul National University Hospital's Institutional Review Board. Informed consent was obtained from all individual participants included in this study.

Results

Clinical presentations

Of the 17 pediatric patients in this study population, 11 were male and 6 were female. The mean age at onset was 2.3 ± 2.6 (range, 0.1–8.0) years, and the mean age at the last follow-up was 12.1 ± 8.9 (range, 0.3–32.3) years. Three patients had a family history of dRTA. One patient with *ATP6VOA4* mutations had an affected sibling. One patient with a *SLC4A1* mutation and another patient without any genetic mutation had an affected mother. All patients were born after a normal full-term pregnancy, and their birth weights ranged from 2.92 to 4.6 kg, except for one patient without any mutation who was prematurely born at 35⁺³ weeks of gestation. Vomiting (*n* = 5) and urinary tract infection (*n* = 5) were the two most common presenting symptoms of dRTA, followed by short stature/rickets (*n* = 4), failure to thrive (*n* = 3), and incidentally detected nephrocalcinosis (*n* = 1). Of the two patients without a genetic mutation, nephrocalcinosis was detected in one patient on fetal ultrasonography, and his metabolic acidosis became evident at the age of two weeks. The other patient presented with rickets at the age of 1.7 years, and his mother was affected by dRTA and rickets. At initial presentation, 11 patients showed hypokalemia (serum K^+ < 3.5 mEq/L) at the initial presentation: six patients with *SLC4A1* mutations, two patients with *ATP6VOA4* mutations, two patients with *ATP6V1B1* mutations, and one patient without any genetic mutation. In addition, one patient with an *ATP6V1B1* mutation had hyponatremia (serum Na^+ 129 mEq/L). None of the patients with *SLC4A1* mutation had red blood cell abnormalities. SNHL was noted in two patients with *ATP6V1B1* mutations. Medullary nephrocalcinosis was detected in all patients at the time of diagnosis (*n* = 14) or during follow-up (*n* = 3). In one patient with an *SLC4A1* mutation, nephrocalcinosis was detected on the initial renal ultrasonography at the time of diagnosis, but it disappeared on a follow-up ultrasonography taken after 2 years of alkali treatment. All patients were treated with oral potassium citrate with or without sodium bicarbonate. At the last follow-up, three (17.6%) patients showed mild renal impairment (CKD stage 2): one patient with an *SLC4A1* mutation (eGFR 83 mL/min/1.73 m²), one patient with *ATP6V1B1* mutations (eGFR 79 mL/min/1.73 m²), and one patient without genetic mutations (eGFR 72 mL/min/1.73 m²). At the last follow-up, five patients had a height equal to or below a -2 standard deviation score: three patients with *SLC4A1* mutations, one patient with *ATP6VOA4* mutations, and one patient without genetic mutations. However, two of the patients with *SLC4A1* mutations and persistent growth failure were followed up with for short periods only.

Mutational studies

Mutational tests revealed pathogenic mutations in 15 of the 17 patients (88.3%), including *SLC4A1* mutations for AD dRTA in 10 patients (58.8%), *ATP6VOA4* mutations in 3 patients (17.6%), and *ATP6V1B1* mutations in 2 patients (11.8%). In the remaining two patients, no pathogenic mutation was detected in any of the four genes. Six of the mutations

Table 1. Genotypes of patients with distal renal tubular acidosis. ^anovel mutations

Patients	Mutated gene	Mutation 1	Mutation 2
1	<i>ATP6V1B1</i>	c.228G>T, p.R76S	c.368G>T, p.G123V
2	<i>ATP6V1B1</i>	c.1356delT, p.F452Lfs	homozygote
3	<i>ATP6V0A4</i>	c.1558_1561delTTTGinsAC, p.F520Tfs*3 ^a	c.2257 C>T, p.Q753*
4	<i>ATP6V0A4</i>	c.1029+5G>A in intron 10 ^a	c.1181C>A, p.A394D ^a
5	<i>ATP6V0A4</i>	c.2261T>G, p.L754R ^a	c.1890G>A, p.A630T
6	<i>SLC4A1</i>	c.1766G>A, p.R589H	
7	<i>SLC4A1</i>	c.2381A>G, p.Y794C ^a	
8	<i>SLC4A1</i>	c.1765C>T, p.R589C	
9	<i>SLC4A1</i>	c.1766G>A, p.R589H	
10	<i>SLC4A1</i>	c.1766G>A, p.R589H	
11	<i>SLC4A1</i>	c.1765C>T, p.R589C	
12	<i>SLC4A1</i>	c.1765C>T, p.R589C	
13	<i>SLC4A1</i>	c.1765C>T, p.R589C	
14	<i>SLC4A1</i>	c.2704G>A, p.D902N ^a	
15	<i>SLC4A1</i>	c.1765C>T, p.R589C	

Table 2. Comparison of clinical findings between patients with *SLC4A1* mutations (Group 1) and patients with *ATP6V0A4* or *ATP6V1B1* mutations (Group 2). ^aestimated glomerular filtration rate calculated using the bedside Schwartz formula, ^bpatients with a height equal to or below –2 standard deviation score

Variables	Group 1 (n = 10)	Group 2 (n = 5)	P value
Onset age (years)	3.7 ± 2.6 (0.6–8.0)	0.2 ± 0.1 (0.1–0.3)	0.002
Gender (M:F)	7:3	2:3	0.329
Current age (years)	14.0 ± 9.6	9.4 ± 7.5	0.159
Initial laboratory data			
Serum sodium (mEq/L)	137 ± 2	138 ± 10	0.575
Serum potassium (mEq/L)	3.2 ± 0.6	2.9 ± 0.6	0.387
Serum chloride (mmol/L)	111 ± 6	115 ± 13	0.759
Serum bicarbonate (mmol/L)	15 ± 3	10 ± 4	0.013
Blood pH	7.30 ± 0.06	7.20 ± 0.06	0.042
Serum anion gap (mmol/L)	11 ± 4	13 ± 5	0.667
Current laboratory data			
Serum sodium (mEq/L)	140 ± 2	141 ± 3	0.135
Serum potassium (mEq/L)	3.9 ± 0.4	4.4 ± 0.6	0.210
Serum chloride (mmol/L)	107 ± 3	108 ± 4	0.355
Serum bicarbonate (mmol/L)	23 ± 4	24 ± 4	0.711
eGFR ^a (mL/min/1.73 m ²)	107 ± 45	112 ± 36	0.854
Patients with eGFR ≥ 90	9 (90%)	4 (80%)	1.000
Patients with eGFR 60–90	1 (10%)	1 (20%)	1.000
Patients with growth failure ^b	3 (30%)	1 (20%)	1.000

were novel, and two common mutations in *SLC4A1* were observed: p.R589C was detected in five patients and p.R589H was detected in three patients (Table 1). Co-segregation of the biallelic mutations with the phenotype of the family members, including both parents, was confirmed in all five patients with *ATP6V0A4* or *ATP6V1B1* mutations. Among the 10 patients with *SLC4A1* mutations, 8 had a *de novo* mutation and 1 had a mutation inherited from the mother. In the remaining patient, parental samples were unavailable.

Genotype–phenotype correlations

Patients with *SLC4A1* mutations (Group 1) had an older age at onset (3.7 ± 2.6 years; range, 0.6–8.0) compared to other patients (0.4 ± 0.6 years; range, 0–1.7) or patients with *ATP6V0A4* or *ATP6V1B1* mutations (Group 2; 0.2 ± 0.1 years; range, 0.1–0.3). At the initial presentation, patients with *SLC4A1* mutations showed less severe acidosis (higher serum bicarbonate and blood pH levels) than other patients. Group 1 patients had higher initial serum potassium levels than Group 2 patients but without statistical significance (Table 2). Clinical features at initial presentation in patients with *ATP6V1B1* mutations and patients

with *ATP6V0A4* mutations were similar, although the number of patients in both groups was very small. At the last follow-up, no statistically significant difference was observed in the renal outcome or the rate of failure to catch-up growth according to the genotypes of the patients (Table 2).

Discussion

Several comprehensive genotype–phenotype correlation studies of patients with dRTA have been published worldwide [10, 13, 15]. In this Korean cohort study of pediatric patients with dRTA, the most distinctive finding compared to other studies was the high incidence (58.8%) of patients with *SLC4A1* mutations (Table 3). In one previous study [10] including eight Asian patients, only one Asian patient had compound heterozygous *SLC4A1* mutations. All of the 10 patients with *SLC4A1* mutations in our study had the AD form of dRTA, which is different from the AR dRTA that is prevalent in Southeast Asian countries [7, 8]. In a small-scale study in China [12], which is a neighboring Far East country, none of the four index patients with dRTA had *SLC4A1* mutations. However, a study from Japan [14], another neighboring country, screened only *ATP6V1B1* and *ATP6V0A4* and showed that five of the nine index patients had no mutations in either of the genes and that at least some of these five patients are likely to have *SLC4A1* mutations.

In the current study, we found six novel mutations: four in *ATP6V0A4* [c.1558_1561delTTTGinsAC (p.F520Tfs*3), c.1181C>A (p.A394D), c.2261T>G (p.L754R), and c.1029+5G>A in intron 10] and two in *SLC4A1* [c.2381A>G (p.Y794C) and c.2704G>A (p.D902N)]. Although we did not perform any functional studies, all four novel missense mutations were predicted to be pathogenic by web-based programs, including PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (<http://sift.jcvi.org/>), and Mutation Taster (<http://www.mutationtaster.org/>). The allele frequency of the c.1029+5G>A variation (rs147476317) in *ATP6V0A4* is reported to be 0.00002514 on the ExAc Browser (<http://exac.broadinstitute.org/>). Although we did not perform any functional studies for this variation, web-based programs, including NetGene2 (<http://www.cbs.dtu.dk/services/NetGene2/>), BDGP (<http://www.fruitfly.org/>) and GENSCAN 1.0 (<http://genes.mit.edu/GENSCAN.html>) predicted this variation to cause abnormal splicing.

In this study, patients with *SLC4A1* mutations presented with milder metabolic acidosis with an older onset age than patients with *ATP6V1B1* or *ATP6V0A4* mutations. However, the clinical courses and the long-term prognosis showed no difference between the two groups. These findings were consistent with those of previously reported studies [10, 13, 15].

The p.R589H in *SLC4A1*, the most common mutation causing AD dRTA, was shown to exhibit only mild reduction in anion-exchange activity [18]. One study [19] showed that retention of heterologous AE1 p.R589H in the endoplasmic reticulum of polarized Madin-Darby canine kidney cells, along with co-retention of co-expressed wild-type AE1, suggesting dominant negative trafficking defects. However, a recent study [20] with an Ae1

Table 3. Underlying genetic defect in patients with distal renal tubular acidosis compared to that in other studies. ^apatients who have a heterozygous mutation(s) in one or two (digenic) of the genes

Studies (No. of index cases)	Origin of patients	Mutated genes			Heterozygotes ^a	No mutation
		<i>ATP6V1B1</i>	<i>ATP6V0A4</i>	<i>SLC4A1</i>		
Pakizzo et al (n = 89) [10]	Italian 70	25 (28%)	30 (38%)	9 (10%)	7 (8%)	18 (20%)
Besouw et al (n = 23) [11]	Asian 8, Caucasian 7	6 (26%)	9 (39%)	4 (17%)	1 (4%)	3 (13%)
Gómez et al (n = 10) [12]	Caucasian 7	1 (10%)	4 (40%)	3 (30%)	1 (10%)	1 (10%)
Elhayek et al (n = 20) [13]	Tunisian 20	13 (65%)	5 (25%)	0	2 (10%)	0
Gao et al (n = 4) [14]	Chinese 4	2 (50%)	2 (50%)	0	0	0
Miura et al (n = 9) [15]	Japanese 9	2 (22%)	2 (22%)	Not tested	0	5 (56%)
This study (n = 17)	Korean 17	2 (12%)	3 (18%)	10 (59%)	0	2 (12%)

p.R607H knock-in mouse model (which corresponds to human AE1 p.R589H) showed that both heterozygous and homozygous knock-in mice exhibited incomplete dRTA (less severe in heterozygotes than in homozygotes). Interestingly, the targeting of Ae1 p.R607H to the basolateral plasma membrane of the α -intercalated cells was normal, as was transport activity *in vivo*. In addition, impaired targeting of the H⁺-ATPase and fewer α -intercalated cells indicated underlying impaired acid secretion in both heterozygous and homozygous knock-in mice. In the present study, none of the patients, including three patients carrying dominant p.R589H mutations, had incomplete dRTA. In addition, the clinical and laboratory findings of the patients with p.R589H mutations were not significantly different from the patients carrying other *SLC4A1* mutations (data not shown).

SNHL is also often associated with dRTA, primarily in patients with *ATP6V1B1* or *ATP6VOA4* mutations. Previous studies have shown that *ATP6V1B1* mutations typically cause dRTA with SNHL, whereas *ATP6VOA4* mutations are responsible for dRTA in patients without SNHL or with late-onset hearing loss [4, 5]. However, this concept was challenged by later studies that demonstrated genetic heterogeneity in dRTA associated with SNHL [21, 22]. Therefore, it is currently believed that the presence or onset age of SNHL cannot discriminate between *ATP6V1B1* and *ATP6VOA4* mutations. In a recent large cohort study [15], the frequencies of the accompanying SNHL in patients with *ATP6V1B1* and *ATP6VOA4* mutations were 92% and 56.7%, respectively. In addition, SNHL in patients with *ATP6V1B1* mutations showed an earlier onset than SNHL in those carrying *ATP6VOA4* mutations. However, SNHL in the latter group of patients presented a wider age range of clinical onset of SNHL (range, 2–552 months), encompassing infancy and early childhood. Notably, one patient with a homozygous *SLC4A1* mutation in the study had been diagnosed with SNHL at 12 years of age; however, the causal relationship between the genetic defect and the development of SNHL remains unclear. These findings warrant the long-term follow-up of hearing in children with dRTA, normal results in an initial hearing screening, and mutations in any of the genes or in whom no mutation is detected. In our study, SNHL was noted in both patients with *ATP6V1B1* mutations only, and no other patient developed SNHL during follow-up.

Medullary nephrocalcinosis is one of the characteristic findings of dRTA. In patients with chronic acidosis, excessive bone mineralization results in hypercalciuria, which, in turn, can result in nephrocalcinosis and/or nephrolithiasis. In our study, all patients had nephrocalcinosis. Interestingly, nephrocalcinosis was detected on fetal ultrasonography in one patient without a genetic mutation, and it disappeared after treatment in another patient with an *SLC4A1* mutation.

Some patients with dRTA present hypokalemia and salt-losing nephropathy [2]. Because dRTA is genetically a single-cell disease involving only the α -intercalated cells, these symptoms are not explained easily, as they are not directly related to the α -intercalated cell functions. Gueutin et al. [23] demonstrated that inactivation of the B1 subunit of the apical H⁺-ATPase not only affects the α -intercalated cells, causing a defect in acid secretion as expected, but also impairs Na⁺ and Cl⁻ reabsorption along the cortical collecting ducts. This impairment occurs by primarily diminishing the function of the β -intercalated cells and thus, secondarily, affecting the principal cells as well. In addition, defective water and salt reabsorption along the cortical collecting ducts causes hypokalemia associated with dRTA. Therefore, dRTA should be reconsidered as a renal disease involving different renal epithelial cell types. In this present study, 11 patients showed hypokalemia and one of the patients also had hyponatremia at the initial presentation. However, there was no genotype–phenotype correlation in the serum potassium and sodium levels at the initial presentation.

Patients with dRTA may develop CKD during long-term follow-up, which could be explained by the combination of nephrocalcinosis and persistent hypokalemia, leading to progressive tubulointerstitial damage, or by kidney damage following repeated episodes of dehydration and acute kidney injury. A large cohort study showed CKD in 32.3% of all patients (31.3% patients with pathogenic mutations) [15], and another study observed CKD in 37.5%

of all patients (36.8% patients with pathogenic mutations) [10]. Most of the patients with CKD in these studies were followed up with for at least 15 years or longer. In our study, CKD was observed in 17.6% of the total patients (13.3% of patients with pathogenic mutations). The lower frequency in our study may be attributed to the shorter follow-up period.

Growth failure and/or rickets, resulting from excessive bone mineralization in association with chronic metabolic acidosis, are another characteristic feature of untreated patients with dRTA. However, catch-up growth is observed in most patients after the initiation of alkaline treatment [24]. In a recent study [10], growth retardation with a height below a -2 standard deviation score was found in 12 of the 24 patients at presentation but persisted in only three of these children once alkali treatment was initiated. In our study, at the last follow-up, five patients had persistent growth retardation: three patients with *SLC4A1* mutations, one patient with *ATP6V0A4* mutations, and one patient without genetic mutations. However, two of the patients with *SLC4A1* mutations were followed up for very short periods to evaluate catch-up growth. All patients with persistent growth retardation had normal eGFR, except for one patient without genetic mutations whose current eGFR was 72 mL/min/1.73 m².

Conclusion

In the genotype–phenotype correlation study of 17 Korean pediatric patients with dRTA, we found that 1) *SLC4A1* was the most common defective gene, 2) patients with *SLC4A1* mutations showed milder metabolic acidosis with older onset age, and 3) there was no genotype–phenotype correlation in the long-term prognosis. Although dRTA is generally accepted as a benign disorder when adequate alkali therapy is initiated early in life, our results suggest that long-term follow-up is necessary to monitoring hearing ability, renal function, and growth.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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