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Molecular Aspects of Distal Kidney Tubular Acidosis in Children, Its Long-Term Outcome, and Relationship with Hyperammonemia

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What is already known on this topic?

- *Kidney tubular acidosis (KTA) defines a group of diseases accompanied by metabolic acidosis with normal serum anion gap.*
- *There are 3 major forms of the disease. The main cause of acidosis in distal KTA is the insufficiency of H^+ ion secretion and HCO_3^- absorption in type A intercalated cells in the collecting tubules.*
- *Inherited distal KTA occurs with mutations in 5 genes:* ATP6V0A4, ATP6V1B1, SLC4A1, *and 2 further genes* FOXI1 *and* WDR72.

What this study adds on this topic?

- *Different clinical and biochemical findings can be seen in patients with dKTA with different mutations.*
- *We present the clinical features, molecular diagnosis, and the prognosis of 9 children with dKTA. We have found 8 different types of mutations, and 2 of them were novel providing valuable data for genotype–phenotype evaluation.*
- *Distal kidney tubular acidosis may be associated with hyperammonemia. This diagnosis should be included in the list of causes of hyperammonemia in children and this phenomenon may be more common than reported. We recommend keeping potassium levels at high-normal levels to reduce ammonia levels, especially in the absence of acidosis.*

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ABSTRACT

Objective: We aimed to present the characteristics, genetic analysis results, long-term prognosis of our patients with distal kidney tubular acidosis, and the relationship between hyperammonemia and distal kidney tubular acidosis.

Materials and Methods: Biochemical, clinical, and imaging findings were collected at presentation and the last clinic visit, and results of the genetic analysis were recorded.

Results: Our study included 9 patients (3 female, 33%). The median age at diagnosis was 3 months, and the median follow-up period was 111 months. Height standard deviation scores were less than −2 in 4 (44%) patients at presentation and in 3 (33%) at the last clinic visit. The median estimated glomerular filtration rate was 98 mL/min/1.73 m^2 at presentation and 126 mL/min/1.73 m^2 at the last clinic visit. We have found 8 different types of mutations of 2 genes, including 6 in the *ATP6V0A4* gene, 2 in the *SLCA4A1* gene, and 2 of them were novel. At the time of presentation, nephrocalcinosis and hypercalciuria were present in all our patients, but at the last visit, only 1 patient had hypercalciuria. Sensorineural hearing loss was found in 4 of our patients with a mutation in the *ATP6V0A4* gene. Serum ammonia levels were found to be high in 3 patients with mutations in the *ATP6V0A4* gene.

Conclusion: Adequate metabolic control is essential for optimal growth and preserved kidney function in distal kidney tubular acidosis patients. Distal kidney tubular acidosis may be associated with hyperammonemia. We recommend keeping potassium levels at high-normal levels to reduce ammonia levels, especially in the absence of acidosis.

Keywords: Distal kidney tubular acidosis, genetics, children, hyperammonemia

INTRODUCTION

Kidney tubular acidosis (KTA) is defined as a group of diseases accompanied by metabolic acidosis with a normal serum anion gap in which bicarbonate (HCO_3^-) absorption alone or with hydrogen ion (H^+) excretion from kidney tubules is affected.[1] There are 3 major forms of the disease. The main cause of acidosis in distal KTA (dKTA), also called type 1 KTA, is the insufficiency of H^+ ion secretion and HCO_3^- absorption in type A intercalated cells in the collecting tubules.[2] Proximal KTA, also called type 2 KTA, results from problems in the reabsorption of filtered HCO_3^- from the proximal tubules. In hyperkalemic KTA (type 4), H^+ ion and potassium (K^+) secretion in the collecting tubules and ammonium excretion are insufficient.

The etiology of dKTA can be genetic or acquired secondary to autoimmune diseases, uropathies, or drugs.[3–5] Inherited dKTA occurs with mutations in 5 genes: *ATP6V0A4, ATP6V1B1, SLC4A1* and 2 further genes *FOXI1* and *WDR72*. Mutations in α-intercalated cells (type A) in

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collecting tubules constitute the most important genetic causes of dKTA. Autosomal recessive mutations in *ATP6V0A4* and *ATP6V1B1* genes cause dKTA. *SLC4A1* encodes anion exchanger 1 (AE1) located in the basolateral cell membrane of α-intercalated cells. Several mutations in the *SLC4A1* gene are associated with the autosomal dominant and recessive forms of the disease. Biallelic recessive mutations of forkhead transcription factor (*FOXI1*) can cause dKTA by disrupting cell function at the transcriptional regulatory level.[6] Pathogenic variations in tryptophan-aspartate repeat domain 72 (WDR72) gene have an effect in the pathogenesis of dKTA, that its protein product is associated with intracellular acid-base regulatory proteins, may also be a cause of hereditary dKTA.[7]

Typical findings of children with dKTA are growth retardation, polyuria, polydipsia, vomiting, diarrhea or constipation, and rickets. Biochemical features are normal anion gap metabolic acidosis, hypokalemia, hypercalciuria, hypocitraturia, inability to lower urine pH below 5.3-5.5, and positive urine anion gap despite metabolic acidosis.[8,9] Radiological features are nephrocalcinosis, nephrolithiasis, and improperly treated patients may have radiological findings of rickets and osteomalacia.[8]

Different clinical and biochemical findings can be seen in patients with dKTA with different mutations. Mutations in the *ATP6V0A4* and *ATP6V1B1* genes can also cause sensorineural hearing loss (SHL), but the age of onset of SHL is different between these 2 types of gene mutations. Hearing loss is usually clinically detectable during adolescence with the *ATP6V0A4* gene mutations, whereas with the *ATP6V1B1* gene mutations, it occurs before puberty.[8,10] In addition, patients with the autosomal recessive form of the SLC4A1 mutations may also have hemolytic anemia.[11,12]

Ammonia metabolism may also be affected in dKTA, and this diagnosis should be included in the list of causes of hyperammonemia in children. Increased ammonia synthesis in response to metabolic acidosis, chronic hypokalemia, and impaired passage of ammonia into the acidified urine result in increased renal ammonia production and ammonia reabsorption leading to serum hyperammonemia in dKTA.[13]

Distal kidney tubular acidosis is a rare disorder, and in this study, we aimed to present the characteristics, genetic analysis results, long-term prognosis of our patients with dKTA, and the long-term effects of the disease, especially on growth. We also examined the possible relationship between hyperammonemia and dKTA and discussed management options for hyperammonemia in dKTA.

MATERIALS AND METHODS

Patients and Biochemical Analysis

Nine patients with dKTA followed in our pediatric nephrology clinic were included in this study. At presentation, all patients except P1 who were previously diagnosed in another center and were on treatment had hypokalemia and hyperchloremia with normal anion gap metabolic acidosis. Therefore, P1 was excluded from the calculation of the initial mean values of the biochemical parameters. All patients had genetic analysis and had significant mutations that caused dKTA. Age at diagnosis, presenting symptoms, gender, follow-up time, growth parameters, estimated glomerular filtration rates (eGFRs) at diagnosis and the last visit, laboratory analysis results, imaging studies, and medical treatments were recorded. Estimated GFR was calculated by the Schwartz formula.[14] Renal ultrasonography was performed at presentation in all children and repeated in follow-up. Hypokalemia was defined as a serum potassium level below 3.5 mmol/L and hyponatremia as a serum sodium level below 135 mEq/L.

Estimated glomerular filtration rates at diagnosis and the last visit were measured. To examine the effect of metabolic control on eGFR changes, the percentage of visits in which patients had adequate metabolic control was also calculated. Adequate metabolic control was defined as normal serum bicarbonate level (>22.0 mmol/L), normal serum potassium level (>3.5 mmol/L), normal serum sodium level (>135 mEq/L), and normocalciuria. Normocalciuria was defined with the normal ranges for the patient's age.[15] The correlation between the eGFR at the last visit and the percentage of visits with adequate metabolic control was assessed. We finally evaluated the correlation between the change in percentages of the eGFRs (at diagnosis and the last visit) and the percentage of visits with adequate metabolic control.

Assessment of Growth

Body height standard deviation scores (SDS) were recorded at the presentation and the last visit according to the anthropometric references in Turkish children.[16] Severe growth failure was defined as a height SDS less than −2. To evaluate the effect of metabolic control on growth, the correlation between the height SDS at the last visit and the percentage of visits with adequate metabolic control was assessed. Lastly, we evaluated the relationship between the change in percentages of the height SDS (at diagnosis and the last visit) and the percentage of visits with adequate metabolic control.

Serum Ammonia Levels

Serum ammonia levels were examined at least once in the follow-up, and simultaneous bicarbonate and potassium levels were recorded. In patients with high serum ammonia levels, other causes of hyperammonemia such as organic aciduria, urea cycle defects, fatty acid oxidation defects, and impaired liver ammonia metabolism were excluded. Then, if there were metabolic abnormalities such as hypobicarbonatemia or hypokalemia that could cause an increase in serum ammonia level, these underlying causes were treated. Ammonia-lowering treatment was started in patients whose serum ammonia levels persisted above 50 μmol/L.

Genetic Analysis

Detailed pedigree analysis and written informed consents were obtained. All patients' total genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). *ATP6V0A4* (NM_020632), *ATP6V1B1* (NM_001692), and *SLC4A1* (NM_000342.4) were sequenced using Sophia Nephropathies Solution (NES) kit via next-generation sequencing [(NGS), Illumina Nextseq 500]. Single nucleotide variants and copy number variations were analyzed through Sophia-DDM-v4 platform. Rare variants with minor allele frequency <1% according to population

studies [1000 Genome (1000G), genome aggregation database (gnomAD), ESP, and ExAC] were filtered. Retained variants were searched in Clinvar and Human Gene Mutation Database (HGMD). Provean, Mutation taster, Polyphen, SIFT, and Human splicing finder (HSF) in silico tools were used to predict the pathogenicity of the detected variants. Segregation analysis for detected pathogenic variants was done with ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, Calif, USA). Hyperammonemia-related genes *(SLC22A5, UQCRB, TMEM70, HMGCL, CPT2, CA5A, HLCS, ASL, SLC25A20, CYC1, SERAC1, GLUL, MCCC2, ATPAF2, CPT1A, MMAA, ARG1, MMAB, MCCC1, BCS1L, MUT, TTC19, ASS1, TUFM, HADHB, GLUD1, HADHA, SLC25A15, BTD, SLC7A7, CPS1, PCCA, PCCB, UQCRQ, LYRM7, UQCRC2, NAGS, OTC, and SLC25A13)* were also sequenced at NGS platform.

This study was approved by the local ethics committee of the Marmara University hospital (Protocol number: 08.09.2021.1143). The study protocol was described to the parents of all children, and informed consent was obtained from the parents of all children included in the study.

Statistical Analysis

R-Studio 4.0.3 was used for statistical analysis. Descriptive statistics were expressed as mean with standard deviation (mean ± SD) in case of normal distribution and median and minimum-maximum values (min-max) in case of non-normal distribution. Categorical variables were expressed as numbers and percentages. The normality of distribution for continuous variables was confirmed by the Shapiro–Wilk test. Wilcoxon test was used to evaluate variables between pre- and post-terms. Spearman's correlation was used for the relationship between continuous variables. Pearson's correlation was used for the relationship between variables in case of normal distribution. For all statistical comparisons, a *P* value below .05 was assumed as statistically significant.

RESULTS

Patients and Biochemical Analysis

Nine patients (3 female, 6 male) with dKTA were included in this study. Demographic findings and biochemical parameters at presentation are summarized in Table 1. The median age at diagnosis was 3 months (min-max 1-18 months). Six consanguineous marriages were found in 9 families. The median follow-up time was 111 months (min-max: 19-221 months) (Table 2). The most frequent complaints were vomiting in 3 (33.3%), growth failure in 2 (22.2%), and polyuria–polydipsia in 2 (22.2%) patients (Table 1). At the time of presentation, the mean values of serum sodium and potassium were 138 ± 8.02 mEq/L and 2.8 ± 0.73 mEq/L, respectively. Two of eight patients (25%) had hyponatremia and 6 of 8 patients (75%) had hypokalemia at the time of presentation. Mean serum bicarbonate and chloride concentrations were 13.4 ± 4.86 mEq/L and 112 ± 8.99 mg/dL, respectively (Table 1). At the time of diagnosis, patient P6 had serum chloride level in the upper limit of normal and serum potassium level in the lower limit of normal, but this patient later developed more pronounced hyperchloremia and hypokalemia that required potassium replacement. Patient 9 had also normal serum chloride, potassium, and HCO_3 levels at diagnosis. In the follow-up, these values did not deteriorate

Table 1. Clinical,D, and Biochemical Characteristics of the Patients at Presentation

	Gender	Age at Diagnosis (Months)	Presenting Symptoms	sNa (mEq/L)	sK (mEq/L)	sCl (mg/dL)	sHCO$_3$ (mEq/L)	Height SDS at Diagnosis	eGFR (mL/min/1.73m^2) at Presentation	UCa/Cr* (mg/mg) at Diagnosis
P1†	M	2	Vomiting	133	2.3	104	31.4	−2.44	85	1
P2	F	3	Vomiting	126	2.2	116	18.4	−2.78	227	1.2
P3	F	1	Weight loss	152	2.1	128	10	−2.05	24	1.85
P4	M	1	Growth failure	143	2.9	116	9.3	−1.16	58	0.9
P5	M	9.4	Confusion	143	1.8	114	8	−4.41	123	0.9
P6	M	2	Vomiting	131	3.7	109	15.3	−1.54	107	1.9
P7	F	3	Growth failure	137	2.9	115	14.9	−1.66	97	0.9
P8	M	27	Polyuria, polydipsia	137	3.3	112	13.1	0.42	240	1.8
P9	M	108	Polyuria, polydipsia	142	3.7	106	22.1	−1.48	98	0.5

s, serum; F, female; M, male; U, urine; eGFR, estimated glomerular filtration rate; SDS, standard deviation score.
*Age-dependent reference values for urinary Ca/Cr ratio: <1 year: 0.78; 1-2 years: 0.53; 2-3 years: 0.5; 3-5 years: 0.39; 5-7 years: 0.28; 7-17 years: 0.25; †Patient who was previously followed up in another hospital.

Table 2. Estimated Glomerular Filtration Rates (eGFRs), Height SDS, Urinary Ca/Cr Ratio, Medications, and Extrarenal Findings at the Last Visit

Patients	Age at the Last Visit (Months)	Duration of Follow-Up (Months)	Height SDS Score at the Last Visit	eGFR (mL/min/1.73 m²) at the Last Visit	UCa/Cr* (mg/mg) at the Last Visit	Nephrocalcinosis	SHL	Medication at the Last Visit
P1⁺	37	35	0.14	152	0.25	✓	×	Potassium supplementation, bicarbonate, indomethacin, Shohl' solution
P2	150	147	−1.61	158	0.20	✓	✓	Potassium, calcium, and phosphate supplementation, bicarbonate
P3	151	150	−3.12	126	0.18	✓	✓	Potassium, calcium, and phosphate supplementation, bicarbonate
P4	137	136	−4.12	104	0.30	✓	×	Potassium supplementation, bicarbonate
P5	205	111	−3.44	105	0.11	✓	✓	Potassium supplementation
P6	98	96	−0.87	126	0.18	✓	×	Potassium supplementation, bicarbonate, hydrochlorothiazide
P7	224	221	−1.12	125	0.17	✓	✓	Potassium and magnesium supplementation, bicarbonate
P8	128	101	0.81	192	0.10	✓	×	Potassium supplementation, bicarbonate, hydrochlorothiazide
P9	127	19	−1.17	80	0.20	✓	×	Hydrochlorothiazide

⁺Patient who was previously followed up in another hospital.
U, urine; SHL, sensorineural hearing loss; eGFR, estimated glomerular filtration rate; SDS, standard deviation scores.
*Age dependent reference values for urinary Ca/Cr ratio: <1 year: 0.78; 1-2 years: 0.53; 2-3 years: 0.5; 3-5 years: 0.39; 5-7 years: 0.28; 7-17 years: 0.25

and the patient did not need replacement. At the time of presentation, nephrocalcinosis and hypercalciuria were present in all our patients, but at the last visit, only 1 patient (P4) had hypercalciuria (Tables 1 and 2). Medical treatment at the last follow-up included potassium and bicarbonate supplementation in 8 patients, phosphate in 2, magnesium in 1 and calcium in 2, indomethacin in 1, and hydrochlorothiazide in 3 patients (Table 2). All patients with SHL had mutations in the *ATP6V0A4* (NM_020632) gene (Table 2). Hemolytic anemia was not detected in any of our patients.

Estimated GFRs of the patients at the time of diagnosis and the last follow-up are given in Tables 1 and 2. The median eGFR was 98mL/min/1.73m²(min-max:24-240mL/min/1.73m²)atpresentation and 126 mL/min/1.73 m² (min-max: 80-192 mL/min/1.73 m²) at the last clinic visit (Tables 1 and 2). At the last clinic visit, we had only 1 patient with an eGFR <90 mL/min/1.73 m² (P9). But the difference between initial and final eGFRs was not statistically significant ($P = .635$). Also, no correlations were detected between the eGFRs at the last visit/change in percentages of the eGFRs and percentage of visits with adequate metabolic control ($P = .454$ and $P = .431$, respectively).

Assessment of Growth

Height SDS of the patients at the time of diagnosis and the last follow-up are given in Tables 1 and 2. Height SDS were less than −2 in 4 (44%) patients at presentation, however, in 3 (33%) patients at the last clinic visit (Tables 1 and 2). The median height SDS were −1.66 (min/max: −4.41/0.42) and −1.17 (min/max: −4.12/0.81) at the time of diagnosis and at the last visit, respectively. This difference was not statistically significant ($P = .374$). The correlation between the height SDS at the last visit/change in percentages of the height SDS and percentage of visits with adequate metabolic control were also assessed and no statistical differences were detected ($P = .459$ and $P = .326$, respectively).

Serum Ammonia Levels

Serum ammonia levels were high in 3 patients, and all had mutations in the *ATP6V0A4* gene (P1, P3, and P5). The highest

Table 3. The Highest and Lowest Serum Ammonia Values as well as the Accompanying sK^+ and $sHCO_3^-$ Values of the Patients

	sAmmonia (μmol/L)*	sK+ (mEq/L)*	$sHCO_3^-$ (mEq/L)*	sAmmonia (μmol/L)⁺	sK^+ (mEq/L)⁺	$sHCO_3^-$ mEq/L+
P1	80.5	3.7	25.3	30	4.5	25.3
P3	85	3.8	18.5	31	4.1	31.6
P5	217	3.5	31.6	58	4.8	25

s, serum.
*Initial maximum serum ammonia values and sK^+ and $sHCO_3^-$ values which accompany the initial serum ammonia values; ⁺the lowest ammonia values and sK^+ and $sHCO_3^-$ values which accompany the lowest serum ammonia values.

and lowest serum ammonia values as well as the accompanying sK^+ and $sHCO_3^-$ values of our patients are summarized in Table 3.

Genetic Analyses Results

The mutations of the patients are shown in Table 4. In the entire cohort, homozygous mutations in the *ATP6V0A4* gene were detected in 7 patients, and it was the most mutated gene. Six different types of the *ATP6V0A4* (NM_020632) gene mutations were detected, and 1 of them was novel. The novel mutation was homozygous exon 14 deletion in *ATP6V0A4* (NM_020632) gene (P1) (Figure 1). The recurrent mutation was nonsense c.2419C>T (p. Arg807*) mutation in exon 21 detected in 2 patients who were cousins (P2 and P3). Two different types of heterozygous mutations were detected in the *SLC4A1* (NM_000342.4) gene (1 missense and 1 splice-site) in 2 patients and 1 of them was novel. The novel mutation was heterozygous c.349+3G>T splice-site variant in the *SLC4A1* (NM_000342.4) gene (P9) (Figure 2).

DISCUSSION

The present study describes the clinical features and genotypes of 9 children with dKTA from a single center, and it also refers to the association between hyperammonemia and dKTA.

The median age at diagnosis of our patients was 3 months which was consistent with the literature.[9] Autosomal recessive disease-causing mutations in the *SLC4A1* gene are very rare, and the onset occurs at early infancy and childhood.[17] Older age of diagnosis is typical for patients with mostly autosomal-dominant *SLC4A1* mutations.[9,18,19] Similarly, in our cohort, 2 patients with *SLC4A1* mutations (P8 and P9) were older at presentation (27 and 108 months, respectively). One patient with ATP6V0A4 mutation (P5) was also diagnosed at the age of 94 months, but it is known that the symptoms started earlier in this patient, the patient had growth failure, and the diagnosis of the patient was delayed.

Table 4. Distal Kidney Tubular Acidosis-Related Gene Analysis Results of Our Patients. Eight Variants with 2 Novel Variants (Shown in Bold)

Patients	Gene Transcript ID	Zygosity	Mutation	Position	Type of Mutation	Reference
P1	*ATP6V0A4* NM_020632	Homozygous	**Exon 14 deletion**	Exon 14	Gross deletion	**This study**
P2∞	*ATP6V0A4* NM_020632	Homozygous	c.2419C>T (p.Arg807*)	Exon 21	Nonsense	Liu et al., 2018[39]
P3∞	*ATP6V0A4* NM_020632	Homozygous	c.2419C>T (p.Arg807*)	Exon 21	Nonsense	Liu et al., 2018[39]
P4	*ATP6V0A4* NM_020632	Homozygous	c.242_243dupTC (p.Glu82Serfs*6)	Exon 5	Frameshift	Stover et al., 2002[22]
P5	*ATP6V0A4* NM_020632	Homozygous	c.1346G>A (p.Arg499His)	Exon 14	Missense	Stover et al., 2002[22]
P6	*ATP6V0A4* NM_020632	Homozygous	c.2257+1G>A	IVS 21	Splice-site	Li et al., 2012[40]
P7	*ATP6V0A4* NM_020632	Homozygous	292-1G>A	IVS 6	Splice-site	Smith et al., 2000[41]
P8	*SLC4A1* NM_000342.4	Heterozygous	c.1765C>T (p.Arg589Cys)	Exon 14	Missense	Bruce et al., 1997[26]
P9	*SLC4A1* NM_000342.4	Heterozygous	**c.349+3G>T**	IVS 5	Splice-site	**This study**

IVS, intervening sequence, ∞ two cousins.

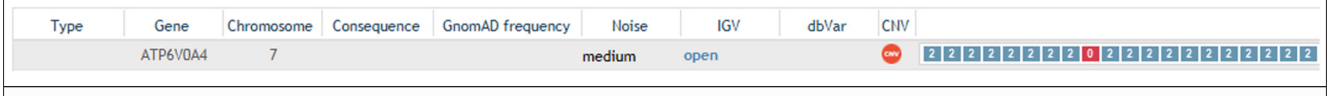


Figure 1. Copy number variation analysis showed homozygous exon 14 deletion in the *ATP6V0A4* (NM_020632) gene (P1).

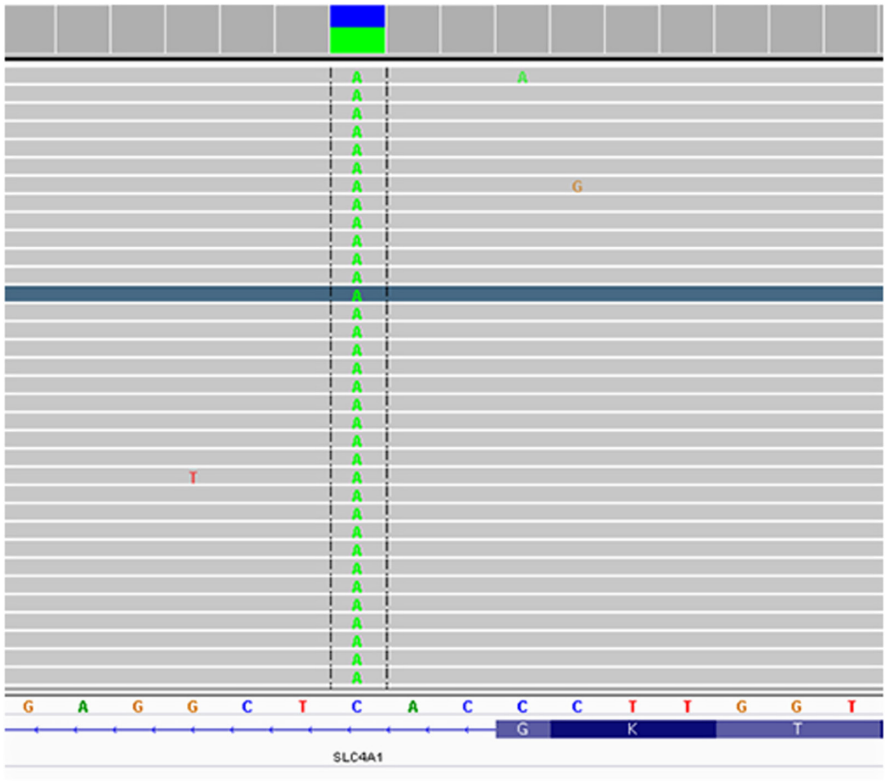


Figure 2. Integrative Genomics Viewer data of novel heterozygous c.349+3G>T mutation in the *SLC4A1*(NM_000342.4) gene (P9)

In our study, 8 different pathogenic mutations were detected in 9 patients diagnosed with dKTA. The most mutated gene was *ATP6V0A4* (NM_020632) with a frequency of 77% (7/9). The α-intercalated cells (type A) in collecting tubules secrete protons into the tubular lumen through apical vacuolar H+-ATPase (V-ATPase) functionally coupled to the basolateral AE1. *ATP6V0A4* and *ATP6V1B1* genes encode the α4 and β1 subunits of the V-ATPase, respectively.[17,20,21,22] *ATP6V0A4* gene (NM_020632) contains 22 exons and located on chromosome 7. In HGMD, 43 missense/nonsense, 14 splicing, 21 small deletions, 7 small insertions, 2 small indels, 1 gross insertion, and 6 gross deletions were reported in the *ATP6V0A4* gene. In this study, we found 6 different mutations in the *ATP6V0A4* (NM_020632) gene in 7 patients; 1 frameshift, 1 missense, 2 nonsense (same mutation in 2 cousins), 2 splice-site mutations, and 1 gross deletion. The novel mutation was homozygous exon 14 deletion. Although exon 13-14 deletions were reported before,[23] only exon 14 deletion had not been reported. In a study with 39 families, *ATP6V0A4* gene mutations were 2-fold more frequent than *ATP6V1B1* gene mutations, similar to the results of our study.[24] In contrast, in a Tunisian cohort, *ATP6V1B1* gene mutations were reported to be twice as common as mutations in the *ATP6V0A4* gene.[25] We did not detect any *ATP6V1B1* (NM_001692) gene mutations.

SLC4A1 gene (NM_000342.4) was located on chromosome 17q21 and had 20 exons. Mutations in this gene can cause either autosomal-dominant or -recessive dKTA. For the SLC4A1 gene, 38 different mutations related to dKTA were reported in HGMD. Twenty-three of them were missense/nonsense, 6 of them were small deletions, 3 of them were small insertions, and 6 of them were gross deletions. One heterozygous missense mutation, which was previously reported to cause autosomal-dominant dKTA,[26] and one novel heterozygous splice-site mutation were detected in this study. c.349+3G>T variant was not found in gnomAD, and according to American College of Medical Genetics and Genomics (ACMG) classification, this novel mutation is classified as "Likely Pathogenic" with the supporting criteria of PP3 (strong) and PM2 (moderate). It is not present in Clinvar interpretations yet. This might be because it is a novel and de novo mutation affecting only our patient but not his parents (P9). This patient had the oldest presentation age, less severe acidosis, one of the highest sodium and potassium levels, and the lowest urinary Ca/Cr ratio compared to other patients, which may be related to the autosomal-dominant form or the novel mutation detected in the SLC4A1 gene. It is already well known that the autosomal-dominant form of SLCA4 gene mutations is milder than the recessive form.[8] No splice-site mutation reported in HGMD was related to dKTA, so this was the first splice-site mutation in this condition. No homozygous mutation was found in the *SLC4A1* gene in this study, which is generally seen in Caucasians.[27] Mutations were scattered throughout the genes, so there was no hot-spot region in both *ATP6V0A4* and *SLC4A1* genes.

Nephrocalcinosis and nephrolithiasis are 2 typical features of dKTA. Both acute and chronic metabolic acidosis stimulate osteoclast activity resulting in calcium release from bone and hypercalciuria. Metabolic acidosis also decreases the calcium channel TRPV5 expression in the distal convoluted and connecting tubules which results in decreased calcium reabsorption.[28] It is well known that citrate in urine prevents the

formation of renal stones.[28] Metabolic acidosis and hypokalemic state cause decreased renal excretion and increased renal reabsorption of citrate which results in hypocitraturia in patients with dKTA. All patients in our cohort had nephrocalcinosis and hypercalciuria and one also had concomitant hyperoxaluria (P8) and another had hypocitraturia (P9) at the time of presentation, but only one patient (P4) had hypercalciuria at the last visit. Similarly, Park et al[29] reported nephrocalcinosis in all their patients with dKTA. Lopez-Garcia et al[19] reported that the frequency of nephrocalcinosis at diagnosis was 88% in a large dKTA cohort that included 340 cases from 29 countries.

Recent studies have shown that kidney function in children with genetic forms of dKTA may deteriorate during long-term follow-up.[10,30,31] Nephrocalcinosis, tubulointerstitial injury, persistent hypokalemia, or recurrent episodes of hypovolemia have been defined to contribute to chronic kidney disease (CKD) progression in patients with dKTA.[30] Besouw et al[10] reported that 37% of patients with dKTA had eGFR values between 60 and 90 mL/min/1.73 m^2 during long-term follow-up, and they also reported that there was no significant relationship between genetic mutation and kidney function. Similarly, Palazzo et al[18] and Atmis et al[9] reported that eGFR values were <90 mL/min/1.73 m^2 in 31.3% and 28.8% of their patients with dKTA, respectively. In our cohort, 3 of our patients (33%) had an eGFR <90 mL/min/1.73 m^2 at presentation, and all of them had pathogenic mutations in the *ATP6V0A4* gene (P1, P3, and P4), but we had only one patient with eGFR <90 mL/min/1.73 m^2 at the last visit (P9). Although median eGFR increased from 98 mL/min/1.73 m^2 at presentation to 126 mL/min/1.73 m^2 at the final visit, the difference was not statistically significant ($P = .635$). This result may be due to the relatively small number of patients for statistical comparison. The kidney function of patients P1, P3, and P4 improved at the last visit. One of these patients was 1 of the 2 cousins (P2 and P3) of similar age at presentation with the same mutation supporting the different phenotype with the same genotype. Palazzo et al[18] reported that CKD usually occurs after adolescence, although in the present study we had patients in the adolescent age group at the last visit, we did not encounter such a situation.

When untreated, dKTA is known to have a severe impact on growth, and the main goal of treatment is to maintain optimal metabolic control and growth. Although the difference between initial and final height SDS was not statistically significant ($P = .374$), in the present cohort, height SDS of 7 patients improved at the last control (Tables 1 and 2). We had only 2 patients (P3 and P4) with a decrease in the height SDS at the last visit and these patients had the lowest percentage of visits with adequate metabolic control with a percentage of 52% and 40%, respectively. All these reasons make us think that there is a significant relationship between metabolic control and growth, even if we could not prove it statistically.

Progressive SHL in hereditary dKTA is caused by mutations in the *ATP6V0A4* and *ATP6V1B1* genes encoding the α4 and β1 subunits of V-ATPase, respectively, expressed in type A intercalated cells of distal tubule, cochlea, and the inner ear.[24,32] In our cohort, *ATP6V0A4* gene mutation was found in all of our 4 patients with SHL, which is consistent with this pathogenesis.

SLC4A1 encodes AE1 in the basolateral cell membrane of α-intercalated cells, and a longer isoform of AE1 is expressed in red blood cells.[11,28] Autosomal recessive forms of this mutation are more likely to be associated with hemolytic anemia.[11,12] In the present study, our 2 patients had the autosomal dominant form of *SLC4A1* gene mutation, but neither had hemolytic anemia.

Miller and Schwartz[33] reported 1 case with hyperammonemia in dKTA, and since then, many reports on this phenomenon had been published.[13,34-36] Ammonia has a significant role in maintaining the acid–base status. Both chronic metabolic acidosis and hypokalemia increase the activity of the key enzymes of ammoniogenesis and renal ammonia reabsorption resulting in high medullary ammonia concentration and subsequent serum hyperammonemia.[36] In our study, we had 3 patients (P, P3, and P5) with a serum ammonia level above 40 μmol/L. They all had *ATP6V0A4* gene mutations. Hsu et al[36] and Saito et al[37] also reported that hyperammonemia occurs in both types of *ATP6V0A4* and *ATP6V1B1* gene mutations in dKTA. In a systematic review of the literature, hyperammonemia secondary to dKTA was detected in 13 children, and this survey suggested that in dKTA hyperammonemia mainly occurs due to hypobicarbonatemia and rapidly normalizes on alkali therapy.[38] Among our patients with high serum ammonia levels, only one patient (P3) had hypobicarbonatemia. Seracini et al[13] reported a 5-month-old girl whose hyperammonemia lasted despite acidosis correction. They supposed that the long-lasting hyperammonemia, in this case, was due to prolonged metabolic acidosis and hypokalemia. Similarly, our patients' ammonia levels also dropped or normalized when their potassium levels reached high-normal levels. We recommend keeping potassium levels at high-normal levels to reduce ammonia levels, especially in the absence of acidosis.

The most important limiting factor of our study is the relatively small number of patients to explain the genotype–phenotype correlation and the number of patients was not sufficient for statistical comparison. We also have some diagnostic limitations in our study. Biochemical tests of some of our patients were not compatible with KTA. We were unable to obtain the results of some of our patients when their symptoms started. Functional tests to explore the proximal wasting of bicarbonate and the urinary acidification capacity are useful diagnostic tools in RTA. We also could not perform these tests in most of our patients. Although currently the molecular basis of the disease can frequently be discovered by gene analysis, we also performed a detailed genetic validation on all of our patients, which was our strong point with the relatively long duration of the follow-up period. On the other hand, we were unable to investigate the recently found *FOXI1* and WDR72 gene mutations and the functional studies for the newly detected mutations.

CONCLUSION

We present the clinical features, genetic results with 2 novel mutations, and the prognosis of 9 children with dKTA providing valuable data for genotype–phenotype evaluation. Adequate

metabolic control is essential for optimal growth and preserved kidney function. Also, dKTA may be associated with hyperammonemia, and this phenomenon may be more common than reported. We suggest that blood ammonia levels should be measured in all cases with dKTA especially in children with severe hypokalemia to prevent further neurologic sequela.

Ethics Committee Approval: This study was approved by Ethics committee of Marmara University (Approval No: 08.08.2021.1143).

Informed Consent: Verbal and written informed consent was obtained from the patients who agreed to take part in the study.

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