Case Report

A Rare Case of Autosomal Recessive ATP6V0A4 Variant of Distal Renal Tubular Acidosis in a Young Female with Recurrent Nephrolithiasis

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ABSTRACT. Homozygous autosomal recessive distal renal tubular acidosis (dRTA) is a rare entity. The intercalated cells in the collecting ducts are defective in apical proton secretion or basolateral bicarbonate reabsorption, due to mutations in genes encoding for proteins in a4 and B1 subunits of the V-ATPase and the anion exchanger Cl−/HCO−3 (kAE1). This results in decreased ammonium (NH4+) excretion and defective urine acidification. dRTA is characterized by hyperchloremic metabolic acidosis with normal anion gap, hypokalemia, hypercalciuria, hypocitraturia, and nephrocalcinosis. Autosomal recessive dRTA is associated with mutation in ATP6V1B1 (2p13) or ATP6V0A4 (7q34) genes. ATP6V1B1 mutation is associated with early- onset sensory neural hearing loss (SNHL), whereas ATP6V0A4 gene mutation may be associated with early- to late-onset SNHL. We report the case of a 30-year-old married woman diagnosed with dRTA at three months of age with mild SNHL, showing homogygous nonsense mutation in exon 3 of the ATP6V0A4 gene that resulted in a stop codon and premature truncation of the protein at codon 6.

Introduction

Renal tubular acidosis is an underdiagnosed entity characterized by normal anion gap hyperchloremic metabolic acidosis in children, majorly due to an inherited or acquired defect affecting the bicarbonate reabsorption or hydrogen excretion. Congenital and hereditary...
kidney diseases require an insight into the genotypic, phenotypic, and clinical expression by taking a detailed history and analyzing biochemical parameters and physical characteristics to make a diagnosis. Some of these disorders are diagnosed at birth, but most manifest in childhood or in adult life. The most common pediatric forms of RTA are type 1 and 2.

Hereditary distal renal tubular acidosis (dRTA) results from mutations in gene encoding for three proteins namely a4 and B1 subunit of the V-ATPase and the anion exchanger Cl−/HCO3−(kAE1) expressed in the alpha intercalated cells of the collecting duct. The autosomal dominant form is associated with mutations in the gene encoding the basolateral Cl−/HCO3− exchanger (SLC4A), whereas the autosomal recessive form is caused by the mutations in the B1 (ATP6V1B1) or a4 (ATP6V0A4) subunit of the apical H+ ATPase gene.1-3

Here, we describe the case of a 30-year-old woman diagnosed at three months of age with dRTA with homozygous ATP6V0A4 gene mutation and a family history of a younger sibling with a trivial manifestation of dRTA.

Case Report

Informed consent was obtained from the patient before presenting the report.

A 30-year-old married woman, a known case of dRTA, presented to our center five years back with hypercalciuria and hypokalemia. She was born of nonconsanguineous parents at full-term normal vaginal delivery to a 22-year-old G1P0 mother and weighed 2600 g at birth. At seven years of age, she was identified to have bilateral sensory neural hearing loss (SNHL). The patient attained menarche at 15 years and has had regular menstrual cycles since then.

Previously, she has had multiple hospitalizations, with the most recurrent indication being lower urinary tract infection. She was on oral potassium magnesium citrate but with poor compliance. Investigations showed intact parathyroid hormone (iPTH) 26.3 pg/mL, sodium 140 mg/dL, potassium 2.5 mg/dL, chloride 104 mg/dL, and bicarbonate 21 mg/dL. 25 hydroxyvitamin D3 was 16.1 ng/mL, hence she was supplemented with oral cholecalciferol 60,000 IU once a week for six weeks. Laboratory investigations over the years are shown in Table 1, which showed improvement with treatment. Urine pH was measured immediately after voiding to prevent a wrong interpretation. Computed tomography of the kidney, ureter, and bladder showed bilateral normal-sized kidneys with bilateral medullary nephrocalcinosis and mild dilation of the calyceal system. Dual-energy X-ray absorptiometry (DEXA) scans of left hip, left forearm and lumbar spine done at the age of 23 showed osteopenia according to WHO classification with increased risk of fracture. In 2013, she sustained a pathological fracture of the shaft of the right humerus following a trivial fall requiring open reduction and internal fixation.

In 2014, the renal calculi was sent for Fourier transform infrared spectroscopy analysis which reported mixed type of stone composed of dahlilit (73%), amorphous calcium phosphate (11%), calcium oxalate monohydrate (10%), and protein and blood (6%).

Genetic study: Exon 3 of ATP6V0A4 gene was polymerase chain reaction amplified, and the product was sequenced using Sanger sequencing. A homozygous nonsense variation in exon 3 of the ATP6V0A4 gene (chr7:138455977G>A; c.16C>T) that results in a stop codon and premature truncation of the protein at codon 6 (p.Arg6Ter) was detected by new-generation sequencing and was further validated by Sanger sequencing (Figure 1 and Table 2).

Her younger brother currently aged 26 years, with calcium of 9.6 mg/dL, was diagnosed with dRTA at three years of age. He is now being treated with oral potassium citrate, sodium citrate, and citric acid. Ultrasound of the kidney showed no abnormality. Anthropometry is shown in Table 3.
Table 1. Serial biochemical parameters.

| Biochemical parameters | January 2011 | July 2011 | March 2012 | April 2013 | May 2014 | 2015 | March 2017 | September 2017 | January 2018 |
|------------------------|-------------|----------|-----------|------------|----------|------|------------|----------------|-------------|
| Na (mEq/L)             | -           | 140      | 135       | 144        | 139      | 141  | 139        | 139            | 138         |
| K (mEq/L)              | -           | 2.7      | -         | 3.2        | 5        | 3.3  | -          | 3.9            | 3.7         |
| Cl (mEq/L)             | -           | 104      | -         | 110        | 104      | 108  | 106        | 106            | 100         |
| HCO3⁻ (mEq/L)          | 16.4        | 21       | 23        | 17         | 19.9     | 15   | 24         | 24             | 27          |
| Ca²⁺ (mg/dL)           | -           | 8.8      | 8.2       | 9.2        | 8.7      | 9.8  | -          | 9.1            | 9.5         |
| Magnesium (mg/dL)      | 1.6         | -        | 2.6       | 2.6        | 2.1      | 2.4  | -          | 2.4            | 2.3         |
| Phosphorus (mg/dL)     | -           | 3.2      | 4.9       | 3.1        | 4        | 3.4  | 3          | 3              | 3.6         |
| Creatinine (mg/dL)     | -           | 0.8      | -         | 0.9        | 0.7      | 0.87 | 0.84       | 0.8            | -           |
| Vitamin D              | 26.3        | 16.1     | 19.1      | 18.6       | 12.4     | -    | 34.6       | -              | -           |
| iPTH (ng/L)            | 26.3        | -        | 26.7      | 10.6       | -        | -    | 14.6       | -              | 15.2        |
| Albumin (g/dL)         | -           | -        | -         | -          | 3.4      | 4.9  | 4.3        | 4.3            | -           |
| Uric acid (mg/dL)      | -           | -        | -         | -          | 3.2      | 3.6  | 3.5        | 3.5            | -           |
| Blood pH               | 7.24        | -        | 7.41      | 7.30       | -        | -    | 7.42       | -              | -           |

Na: Sodium, K: Potassium, Cl: Chloride, HCO: Bicarbonate, Ca: Calcium, Vit D: Vitamin D, iPTH: Intact parathyroid hormone.

Figure 1. Sequence chromatogram and alignment to the reference sequence showing the variation in exon 3 of ATP6V0A4 gene (chr7:138455977G>A; c.16C>T; p.Arg6Ter) detected in homozygous condition.
Discussion

Genetic study involving dRTA has not been reported previously from India. Our patient was diagnosed with dRTA in early infancy, with SNHL identified at seven years and was found to have homozgyous nonsense mutation in the ATP6V0A4 gene on evaluation. Unfortunately, all the offsprings will be carriers, due to homozygous mutation of the gene. Mutation in the ATP6V0A4 gene encoding four subunits of the V-ATPase results in recessive dRTA associated with variable SNHL. As dRTA is not an uncommon entity in India, unless a detailed family history, biochemical, and genetic studies are undertaken with longitudinal follow-up as in our patient, many undiagnosed patients may not receive appropriate counseling and treatment. In adults, 1–2 mEq/kg of sodium bicarbonate is recommended to maintain serum bicarbonate concentration above 22 mmol/L as the defect in dRTA is a hydrogen excretion defect. Compliance should be ensured by following up the biochemical parameters, as our patient had metabolic acidosis during the periods of noncompliance and mildly stunted growth. In the absence of alkali therapy, progressive hydrogen ion retention leads to a fall in plasma bicarbonate concentration that is accompanied by an abnormally high urine pH (>5.3) as in our patient. This often results in growth retardation. Hence, it is of paramount importance to initiate alkali therapy early and maintain levels above 22 mmol/L. Plasma potassium is usually reduced,

Table 2. Primers used for polymerase chain reaction DNA amplification of the ATP6V0A4 gene.

| Exon | Forward | Reverse | Annealing temperature |
|------|---------|---------|-----------------------|
| Exon 3 | TCACCCTAAGGTTTTTCAC | ACCATAAAGAAGTGTTGTC | 55°C |
| Exon 4-5 | TGCCCTCCAGTCTGGGTGAC | AAGTGCTCTCAGGGCTTTC | 65°C |
| Exon 5 | CACATGTCTTTGCTTGAACCTC | TCAAGGAGACAGGCTTTCACTG | 55°C |
| Exon 6 | GTATGTGTATTAGGCTTGC | GAGTGAATTTACTCAGATTC | 56°C |
| Exon 7 | AATCTTGGAGGTAGGTTGCC | ACCGGTCTACTCAGCTAC | 55°C |
| Exon 8 | GGTGTGTCTTACGCTTACCT | TTTGGGAGAAGCAAGCAGG | 55°C |
| Exon 9 | GGAAAGTTACAGTCTTACG | GTCACTGTACTTGTCATGGT | 55°C |
| Exon 10 | AGCAAAACCGATGTCTTACG | GCAGGGTCTTGTAGAAAC | 55°C |
| Exon 11 | AAGCTGCTGTGCTGCTGAC | ATGTCGGCCCATAGGCAAC | 55°C |
| Exon 12 | CTGGGACTGTGACCTTTCG | TCCACCACCTCAGTCACCTC | 55°C |
| Exon 13 | CCAGAAAGCTTAAAGGAAAC | ATGGTGAAAAACACATACAT | 55°C |
| Exon 14 | TATCCGTGAGAACGCTTCCC | GTACACCTAGGTGACTAC | 55°C |
| Exon 15 | ATGGGCAAGTCTGTGACTGG | TTTGACAGGCTTTGTTGGA | 55°C |
| Exon 16 | AAGTTGTGTACTGAGGCCCCTC | GCAGAGAGAGATGCACT | 55°C |
| Exon 17 | GTGCTTTTGTGCTTGTGAGG | AAGAGAGACCTTCTCCTC | 62°C |
| Exon 18 | GGCCTCATAGGTCTTACTT | AGGAGATTTCTCTCCTAAAC | 55°C |
| Exon 19 | GTAGTATTTAGGAGAGC | ACCAGCTCTACTGCTCATG | 55°C |
| Exon 20 | ACTGAAGTATGTGCTGACAGC | GAGAGTCAGGTCTTTATCC | 55°C |
| Exon 21 | ACAGAGTGAGACCTGTGCTC | CTCAACAGATCTGACCCGTC | 55°C |
| Exon 22 | TTTCTGCAACGGCTCTGCTTCC | GAACACCCTTCATTCAGG | 55°C |

Table 3. Anthropometry of the patient, mother, and the affected sibling on initial presentation; however, her current BMI is 25.3 kg/m².

|         | Height (cm) | Weight (kg) | BMI (kg/m²) |
|---------|-------------|-------------|-------------|
| Patient | 145         | 47          | 16.2        |
| Mother  | 161.5       | 57          | 21.9        |
| Brother | 160         | 46.9        | 18.3        |

BMI: Body mass index.
but hyperkalemic forms may rarely exist. Clinically, the etiology of hypokalemia may be broadly divided into the following four groups: pseudohypokalemia, redistribution (certain hormones particularly insulin, aldosterone, and sympathomimetics), extrarenal potassium loss, and renal potassium loss. Currently, our patient is maintaining normal anion gap with no evidence of hypokalemia. At present, she is married and considering pregnancy, hence a genotyping was done. A detailed discussion with an obstetrician suggested high risk in view of nephrocalcinosis and urinary tract infection during pregnancy, which would require close follow-up with a nephrologist and obstetrician. Her low Vitamin D levels, previous history of fracture, and low bone mineral density are also risk factors during pregnancy, and advice from a skilled nutritionist forms an integral part of her management. She had a low BMI initially which on nutritional counseling and correction of metabolic acidosis has improved to 25 kg/m². She requires periodic imaging to assess her nephrocalcinosis and DEXA scans to estimate bone mineral density. Urine anion gap was not measured in our patient with normal anion gap metabolic acidosis as her issue was mainly due to hydrogen ion excretion and not due to ammonia excretion.

Conclusion

dRTA is often underdiagnosed due to the lack of standard laboratory facilities in many parts of India and rarely genetic studies are performed because of the issue with accessibility. This case highlights the importance of early diagnosis, management, and issues related to pregnancy.

Conflict of interest: None declared.

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