

Atypical presentation of distal renal tubular acidosis in two siblings

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Received: 27 December 2007 / Revised: 15 February 2008 / Accepted: 15 February 2008 / Published online: 2 April 2008
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Abstract Primary distal renal tubular acidosis (dRTA) is an inherited disease characterized by the inability of the distal tubule to lower urine pH <5.50 during systemic acidosis. We report two male siblings who presented with severe hyperchloremic metabolic acidosis, high urinary pH, nephrocalcinosis, growth retardation, sensorineural hearing loss, and hypokalemic paralysis. Laboratory investigations revealed proximal tubular dysfunction (low molecular weight proteinuria, generalized hyperaminoaciduria, hypophosphatemia with hyperphosphaturia, and hypouricemia with hyperuricosuria). There was significant

hyperoxaluria and laboratory evidence for mild rhabdomyolysis. Under potassium and alkali therapy, proximal tubular abnormalities, muscular enzymes, and oxaluria normalized. A homozygous mutation in the *ATP6V1B1* gene, which is responsible for dRTA with early hearing loss, was detected in both siblings. In conclusion, proximal tubular dysfunction and hyperoxaluria may be found in children with dRTA and are reversible under appropriate therapy.

Keywords Distal renal tubular acidosis · Hypokalemic paralysis · Rhabdomyolysis · Hyperoxaluria · *ATP6V1B1* mutation

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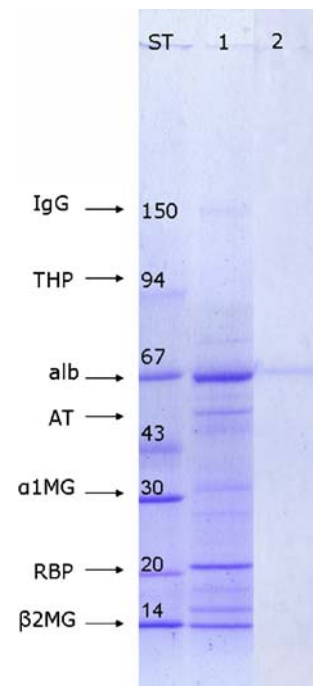
Introduction

Primary distal renal tubular acidosis (dRTA) is an inherited disease characterized by inability of the distal tubule to lower urine pH <5.50 during systemic acidosis. Clinically, these children manifest polyuria/polydipsia, nephrocalcinosis, growth retardation, and metabolic bone disease. Hearing impairment may be found in some patients early or later in life, depending upon which gene is mutated [1–3]. Although rare, hypokalemic paralysis and rhabdomyolysis may ensue [4–8]. Presence of low molecular weight proteinuria (LMWP), hyperaminoaciduria, hypophosphatemia, hypouricemia, and hyperoxaluria may raise confusion related to Fanconi syndrome or primary hyperoxaluria. Herein we report two male siblings with dRTA who presented with atypical laboratory features for the presence of LMWP, hyperaminoaciduria, hypophosphatemia, hypouricemia, and hyperoxaluria. Proximal renal tubular dysfunction and hyperoxaluria normalized in both children under appropriate therapy.

Case report

Sibling 1 A 5.5-year-old boy was referred for investigation due to severe stature and psychomotor retardation. There were deformities of the thorax and lower extremities (crura valga) and rachitic changes on X-ray film of the bones. His body height was 85 cm [−7.5 standard deviation (SD)] and weight 11.5 kg (−3.7 SD). He could not walk due to paralysis of the lower extremities. There were no abnormal tendon reflexes, and sensation tests were also normal. He was deaf. Laboratory investigations showed hyperchloremic metabolic acidosis [Cl 121 mmol/l, pH 7.27, HCO₃ 13.8 mmol/l, base excess (BE) −11.3 mmol/l]. His serum creatinine was 44 μmol/l, and estimated glomerular filtration rate (eGFR) according to Schwartz formula was 94 ml/min per 1.73 m². Serum phosphate was 1.07 mmol/l with tubular phosphate reabsorption (TRP) of 65% (normal >85%). He had normal levels of parathormone (PTH): 43.0 pg/ml (normal 10–65). Serum uric acid was 134 μmol/l, with tubular uric acid reabsorption (TRUA) of 63% (normal >85%). The other abnormalities in serum biochemistry were as follows: potassium (K) 2.7 mmol/l, alkaline phosphatase 344 U/l, creatine kinase (CK) 668 U/l (normal 20–120), aspartate aminotransferase (AST) 74 U/l (normal 14–36), alanine aminotransferase (ALT) 69 U/l (normal 9–52), and lactate dehydrogenase (LDH) 888 U/l (normal 313–618). Urinalysis showed pH at 7.24 (with electrode), protein 1+ (0.66 g/l), low urinary specific gravity (1.005) and osmolality (106 mOsm/kg), and negative tests for glucose, blood, nitrite, and leukocyte esterase. Urinary anion gap (Na+K-Cl) was positive with 33 mmol/l. Urinary solute/creatinine ratios expressed as mmol/mmol were as follows: calcium/creatinine 0.84 (normal <0.70), citrate/creatinine 0.055 (normal 0.150–1.007), oxalate/creatinine 0.445 (normal 0.035–0.126), glycolate/creatinine 0.011 (normal 0.017–0.103). Analysis of urinary proteins with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) revealed LMWP (Fig. 1). Urinary concentration of β₂-microglobulin was increased at 16.33 mg/l (normal <0.14). There was generalized hyperaminoaciduria. Ultrasound scan of the kidneys showed bilateral medullary hyperechogenicity typical for nephrocalcinosis. Audiologic testing revealed severe bilateral sensorineural hearing impairment. The boy was given potassium and alkali therapy. His muscular weakness gradually improved; after 2 months he could stand, and after 3 months he began to walk with assistance. After 3 months of alkali therapy, there was no LMWP (β₂-microglobulin 0.04 mg/l), TRP was 96%, TRUA was 85%, CK 106 U/l, AST 34 U/l, ALT 28 U/l, and LDH 448 U/l. His renal function was normal (serum creatinine 25 μmol/l, eGFR 164 ml/min per 1.73 m²). There was no hyperaminoaciduria. Urinary oxalate excretion also normalized

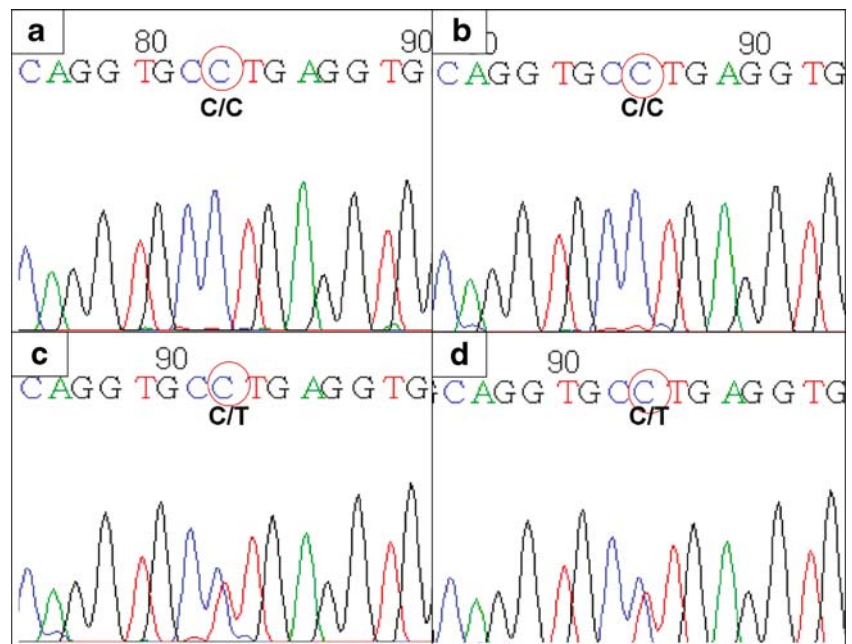
Fig. 1 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis of urinary proteins: low molecular weight proteins (<67 kDa) present on admission (lane 1) but not after 3 months of therapy (lane 2); ST standard, β₂MG β₂ microglobulin, RBP retinol-binding protein, α₁MG α₁ microglobulin, AT antitrypsin, alb albumin, THP Tamm Horsfall protein, IgG immunoglobulin G



(0.072 mmol/mmol creatinine). After 6 months, his growth and weight deficit improved (−5.8 SD and −2.2 SD, respectively). DNA analysis detected a homozygous mutation in exon 3 of the *ATP6V1B1* gene leading to substitution of leucine with proline [⁸¹Leu(CTT)>Pro(CCT)], (Fig. 2).

Sibling 2 This 4.5-year-old male sibling also presented with severe growth and motoric retardation, clinical and radiological signs for rickets, and generalized muscular weakness due to hypokalemia (K 2.9 mmol/l). His body height was 81 cm (−6.5 SD) and weight 11 kg (−3.6 SD). He had hyperchloremic metabolic acidosis (Cl 127 mmol/l, pH 7.20, HCO₃ 9.3 mmol/l, BE −16.7 mmol/l). His serum creatinine was 46 μmol/l and eGFR 86 ml/min per 1.73 m². There was no laboratory evidence for rhabdomyolysis. He had hypophosphatemia (P 1.07 mmol/l) with hyperphosphaturia (TRP 67%) and hypouricemia (145 μmol/l) with hyperuricosuria (TRUA 69%). His PTH level was 57.0 pg/ml. Urinalysis showed pH at 7.16 (with electrode), proteinuria 1+ (0.89 g/l), low urinary specific gravity (1.005) and osmolality (136 mOsm/kg), and negative tests for blood, glucose, nitrite, and leukocyte esterase. The urinary anion gap was positive (35 mmol/l). SDS-PAGE revealed LMWP. β₂-microglobulin was 8.59 mg/l. There was generalized hyperaminoaciduria. He had hypercalciuria (Ca/Cr 1.43, normal <0.70), hypocitraturia (citrate/Cr 0.035, normal 0.150–1.007), and hyperoxaluria (oxalate/Cr 0.243, normal 0.035–0.126), with normal urinary glycolate excretion (glycolate/Cr 0.007, normal 0.017–0.103). Ultrasound scan demonstrated bilateral medullary nephrocalcinosis. Audiologic examination confirmed bilat-

Fig. 2 Mutational analysis of the *ATP6V1B1* gene in the family members. Both siblings were homozygous for c.242T>C mutation in exon 3, causing ⁸¹Leu (CTT)>Pro(CCT), and the parents were heterozygous. (a sibling 1; b sibling 2; c father; d mother)



eral sensorineural hearing loss. Under potassium and alkali therapy, his urinary excretion of phosphate, uric acid, oxalate, amino acids, and LMWP normalized within 3 months. His renal function was normal (serum creatinine 21 $\mu\text{mol/l}$, clearance 187 ml/min per 1.73 m^2 according to Schwartz formula). He began to walk with assistance after 3 months. After 6 months, his gait was stable, he could walk without assistance, and his growth deficit improved (height -4.8 SD, weight -2.0 SD). He was homozygote for the same mutation in the *ATP6V1B1* gene.

Discussion

There are reports of atypical presentation of dRTA, but our patients presented with entirely all unusual/atypical clinical and laboratory features of this disease described so far. The both siblings were referred under suspicion for hypophosphatemic rickets at the age of 5.5 and 4.5 years, respectively. Although they failed to thrive and had polyuria, rickets, and hypokalemia, acid-base status was not evaluated, and they escaped correct and timely diagnosis. Although they had been neglected, both children showed excellent response to treatment in respect of metabolic compensation, improved psychomotor status, and growth parameters. They both showed increased eGFR, which might be explained by the reduction of polyuria and better hydration status. Igarashi et al. reported four patients with dRTA who exhibited increased excretion of LMWP that was reversible with alkali therapy [9]. They believed that hypokalemic proximal tubular damage in untreated dRTA

patients was responsible for LMWP. Recently, Watanabe reported a 4-year-old girl with untreated dRTA who presented with proximal tubular dysfunction including LMWP, hypouricemia with uricosuria, hyperphosphaturia, and generalized hyperaminoaciduria [10]. This patient's TRP normalized after 2 months, LMWP after 1 year, and uric acid and aminoaciduria after 1.5 years. Contrary to Watanabe's patient, our patients normalized all proximal renal tubular abnormalities very quickly—within 3 months of alkali therapy. It is very difficult to find appropriate explanation for proximal tubular abnormalities in children with dRTA. One may suspect defective H^+ -ATPase for impaired endosomal acidification in proximal tubular cells, but this is not accurate, as B1 subunit is not expressed in the proximal tubular cells in contrast to B2 subunit, which retains normal function [11]. Another possibility for proximal tubular dysfunction is long-lasting hypokalemia. Such abnormalities have already been reported in patients with Bartter syndrome [12]. Hypocitratemia and hypercalciuria are explained by metabolic acidosis, whereas generalized aminoaciduria is not a normal feature of acidosis and would support a generalized defect of the proximal tubule due to hypokalemia.

In humans, chronic hypokalemia results in tubulointerstitial injury, which may lead to proximal tubular dysfunction. Emery et al. found that 45% of patients with hypokalemia showed increased urinary excretion of $\beta 2$ microglobulin, which was reversible with potassium supplementation [13]. Hypokalemic nephropathy is characterized with vacuolization of proximal tubular cells; tubular atrophy, destruction, and regeneration of the tubular cells;

and injury of the brush border membrane. Also, there is development of renal cysts, infiltration with mononuclear cells, and tubulointerstitial fibrosis. The precise mechanisms by which hypokalemia induces nephropathy has not yet been clarified, but it is believed that intrarenal hypoxia, as a result of microvascular injury and loss or alterations in local vasoactive mediators, could be responsible for tubulointerstitial injury. In animal studies analyzing chronic K^+ -deficient rats, it was found that intrarenal hypoxia is associated with elevation of renal vasoconstrictive peptides, including endothelin-1 and angiotensin II [14].

At the initial evaluation of our patients, we detected hyperoxaluria. The association between primary hyperoxaluria type 1 and dRTA is extremely rare [15] and was easily excluded in our patients on the basis of its transitory character and normal urinary glycolate excretion. Mehler et al. observed hyperoxaluria in four patients with primary dRTA (one had an incomplete form) [16]. Hyperoxaluria was persistent in all patients. An oxalate absorption test was normal in all patients, which made a secondary reason for hyperoxaluria unlikely. Although the absorption test was negative, they placed their patients on a low-oxalate and high-calcium diet and achieved a significant decrease of urinary oxalate excretion in three patients. Contrary to this report, our patients had transitory hyperoxaluria, which resolved simultaneously with other proximal tubular abnormalities without any specific dietary intervention.

Protein unbound oxalic acid is freely filtered in the glomeruli. In the proximal tubule, it is further secreted and/or reabsorbed, leading to a net oxalic acid excretion of 109–128% in normal renal function. The Cl^- formate/oxalate anion exchanger at the apical renal brush border membrane encoded by a member of the *SLC26* gene family (A6) seems to be the leading transporter of these oxalate excretion processes [17]. The renal proximal tubule secretes oxalate via basolateral *Slc26a1* and apical *Slc26a6*. Bidirectional transport can occur such that a reduction in oxalate reabsorption (rather than secretion) might explain the hyperoxaluria in our patient. Notably, *NaPi2A*, *URAT1*, and *Slc26a6* all bind to the PDZ-domain protein *PDZK1*, such that a reduction in expression of this protein in acidosis might lead to hyperoxaluria, hyperuricosuria, and hyperphosphaturia. Two knockout mice strains deficient in *Slc26a6* have been shown to have hyperoxaluria due to reduced intestinal secretion of oxalate, mediated by *Slc26a6* [18]. Therefore, an alternative explanation is that reduced function of intestinal *SLC26A6* might have led to reduced intestinal secretion and subsequent hyperoxaluria.

Our patients presented with severe weakness and hypokalemic paralysis. This is a rare complication, but in few cases may be fatal due to paralysis of respiratory muscles [6–8]. Acidosis and hypokalemia may produce rhabdomyolysis, another serious complication of dRTA [4, 5, 8]. One of the

siblings had laboratory evidence for mild rhabdomyolysis, which, however, did not lead to any clinical consequences.

Until now, more than 20 mutations in the *ATP6V1B1* gene have been detected in patients with distal renal tubular acidosis and sensorineural deafness [1–3, 19–21]. The homozygous L81P mutation, which was detected in our patients, has also been reported in two other previous reports [1, 18]. The 81st leucine is a highly conserved amino acid during evolution, and substitution of proline for the conserved residue, L81, predicts disruption of an N-terminal β -barrel observed in F1-ATPase [22] and predicted in the corresponding segment of human *ATP6B1* [1].

In conclusion, our patients with dRTA presented with many atypical clinical and laboratory features. Proximal tubular dysfunction and hyperoxaluria may be found in children with dRTA and are reversible under appropriate therapy. All abnormalities normalized very fast in our patients—within 3 months after starting potassium and alkali therapy. We emphasize that for the first time, molecular genetic testing confirmed the diagnosis of dRTA in patients with such a variety of atypical clinical and laboratory features.

Conflict of interest statement None declared.

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