Hypokalemic Distal Renal Tubular Acidosis

Patricia G. Vallés and Daniel Batlle

Distal renal tubular acidosis (DRTA) is defined as hyperchloremic, non-anion gap metabolic acidosis with impaired urinary acid excretion in the presence of a normal or moderately reduced glomerular filtration rate. Failure in urinary acid excretion results from reduced H^+ secretion by intercalated cells in the distal nephron. This results in decreased excretion of NH_4^+ and other acids collectively referred as titratable acids while urine pH is typically above 5.5 in the face of systemic acidosis. The clinical phenotype in patients with DRTA is characterized by stunted growth with bone abnormalities in children as well as nephrocalcinosis and nephrolithiasis that develop as the consequence of hypercalciuria, hypocitraturia, and relatively alkaline urine. Hypokalemia is a striking finding that accounts for muscle weakness and requires continued treatment together with alkali-based therapies. This review will focus on the mechanisms responsible for impaired acid excretion and urinary potassium wastage, the clinical features, and diagnostic approaches of hypokalemic DRTA, both inherited and acquired.

Key Words: Distal RTA, Hyperchloremic metabolic acidosis, Hypokalemia, Growth failure, Acid excretion

INTRODUCTION

The first descriptions of renal tubular acidosis (RTA) were made in children with nephrocalcinosis in 1935 by Lightwood[1] and in 1936 by Butler and colleagues.[2] Later, the term RTA was coined by Pines and Mudge[3] The classical studies of Albright and colleagues[4] characterized the clinical features of the syndrome, documented its association with rickets or osteomalacia, and pointed out to a basic defect in tubular function. This disorder seemed to be relatively rare at first, accompanied with other disturbances of renal tubular transport which were genetically determined in some cases.[5-8] The syndrome, while relatively rare, is of great interest among students of pathophysiology because it was a model of disease in which the biochemical, physiologic, and molecular basis of its pathogenesis could be examined. Unlike adults, in whom RTA is often secondary to acquired causes, children most often have primary forms of RTA. According to their pathophysiological basis, 4 types of RTA were initially categorized.[9-11] Distal type I RTA or classic RTA thereby referred as distal renal tubular acidosis (DRTA) is characterized by reduced net acid excretion and an inability to lower urine pH regardless of the degree of acidemia.[9-14] Proximal type II RTA, by contrast, is characterized by marked HCO_3^- wastage but preserved the ability to lower urine pH when plasma HCO_3^- (and therefore filtered HCO_3^-) is below a certain threshold.[9-11] The term type III RTA was coined to describe patients in whom HCO_3^- wastage coexists with failure to lower urine pH despite profound acidemia.[10-11] The term type IV RTA was introduced by Sebastain and colleagues[12] to designate the type of acidification defect associated with hyperkalemia and attributable primarily to aldosterone deficiency. A hyperkalemic form of DRTA not attributable primarily to aldosterone deficiency was later described.[13-15] This type was also referred to as voltage-dependent RTA to reflect the postulated mechanism that would account for reduced ion secretion, both potassium and hydrogen.[13] This mechanism was postulated based on similarities observed in patients with obstructive uropathy, with the defect observed experimentally in the postobstructed kidney and induced also by the administration of amiloride to block sodium transport.[16,17] As new potential mechanisms were uncovered, the permeability defect theory of DRTA that had been postulated earlier by Seldin and Wilson[18] was no longer tenable as a unique mechanism causing hypokalemic DRTA. Rather, several other potential mechanisms were described.[19-24] Only the alteration in distal acidification caused by amphotericin B, a compound that causes renal potassium wastage and a back leak of protons, demonstrable in epithelial analogs of the mammalian collecting tubule[25-27] has the permeability defect as the mechanism causing DRTA.

Electrolyte disturbances namely hypokalemia or hyperkalemia are key distinctive features of each type of RTA. The clinical phenotype in patients with DRTA associated with hypokalemia is very rich and is characterized by stunted growth with bone abnormalities in children as well as nephrocalcinosis and nephrolithiasis that develop as the consequence of hypercalciuria, hypocitraturia, and relatively alkaline urine. Unique systemic manifestations such as deafness are found in inherited types in which the defect in transporting hydrogen ions is due to mutations in a V-ATPase present in the ear and the kidney collecting tubule. The hyperkalemic forms of RTA, discussed elsewhere in this issue, are usually acquired and lack a specific clinical phenotype other than that of the underlying kidney disease, and alterations in calcium metabolism usually are not present.[13-15,28-29] Advances in renal physiology and the molecular biology of acid-base transporters have allowed a much better understanding of DRTA in general, particularly

From the Pathophysiology Division, Pathology Department, School of Medicine, National University of Cuyo, Mendoza, Argentina; and Division of Nephrology, Department of Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL.

Financial Disclosure: The authors declare that they have no relevant financial interests.

Support: Grant support to D.B. from R01DK104785.

Address correspondence to Daniel Batlle, MD, Earle Del Greco Levin endowed Professor of Medicine, and Hypertension Division of Nephrology, Department of Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL. E-mail: d-batlle@northwestern.edu

1548-5595/$36.00

https://doi.org/10.1053/j.ackd.2018.05.003

of the hereditary syndromes.[30-47] In this review, we will discuss DRTA, both inherited and acquired, with an emphasis on the pathophysiology of the metabolic acidosis and the development of severe hypokalemia, a striking finding that often brings the patients to medical attention as a result of muscle weakness and paralysis.

Overview of Distal Acidification in DRTA

The site of the nephron responsible for the causation of DRTA is felt to be the late distal convoluted tubule and the collecting tubule. Intercalated cells in these nephron segments are responsible for acid secretion by the α-intercalated cell and bicarbonate secretion by the β-intercalated cell (Fig 1). In α-intercalated cells, urinary acidification involves a combination of energy-dependent proton secretion across the apical surface which is mediated mainly by a hydrogen ATPase and to a lesser extent by a hydrogen potassium ATPase.[48-51] Basolateral chloride-bicarbonate exchange that serves to transport bicarbonate back into the blood occurs via a band-3 protein, AE1, which is also critically important for the process of continued acid excretion via the apical H^+-ATPase and H^+/K^+-ATPase pumps (Fig 1)

DRTA can be attributed to failure of the kidney α-intercalated cells to acidify the urine normally as a result of dysfunction in any of the transporters involved in the overall process of acidifying the urine maximally (Fig 1). In DRTA, urine pH is typically well above 5.5 regardless of the degree of systemic acidosis[9-13,30,31] (Fig 2). As a result of impaired distal H^+ secretion, there is not only a failure to lower urine pH maximally but also, more importantly, the excretion of acid as ammonium and other buffers, collectively referred as titratable acids, is markedly decreased.[30,31,43] Typically, the overall level of kidney function is well preserved, and the glomerular filtration rate is normal or near normal so that the formation of ammonia is not limited by a reduced nephron mass. In fact, the associated hypokalemia should increase ammonia formation in the proximal tubule which perhaps provides some degree of compensation for the metabolic acidosis, but this, to our knowledge, has not been formally evaluated. Even when the urine is alkaline, the amount of bicarbonate in the urine is modest and contributes very little to the overall decrease in net acid excretion in DRTA. The decrease in net acid excretion over time results in the development of a hyperchloremic type of metabolic acidosis, the hallmark of DRTA. Kurtz and colleagues[52] have postulated that the decrease in urine ammonia excretion is associated with increased renal vein ammonia delivery with concomitant HCO_3^- consumption in the urea cycle. This would be in part the consequence of the relatively high pH that prevails in the collecting tubule when proton secretion is diminished.[52]

CLINICAL SUMMARY

- In children, DRTA is most often observed as a primary entity. Growth retardation and bone loss are often present because of chronic metabolic acidosis unless alkaline therapy is initiated early.
- Associated features include nephrocalcinosis, nephrolithiasis, hypercalciuria, and hypocitraturia.
- Patients may suffer from weakness and muscle paralysis due to hypokalemia, a striking feature of DRTA.
- Hearing loss is seen in the autosomal recessive type of DRTA caused by mutations in ATP6V1B1 and ATP6V0A4.

The impaired excretion of both NH_4^+ and titratable acid is generally attributed to the decrease in H^+ secretion by α-intercalated cells in the distal nephron to acidify the urine normally.[33-38] This results from dysfunction in any of the acid-base transporters in the intercalated cell involved in the overall process of acidifying the urine (Fig 1). Enhanced bicarbonate secretion by the β-intercalated cell could theoretically also cause decreased net acid excretion, but no cases of DRTA attributable to this mechanism have been clearly demonstrated to date. This could occur with an abnormal targeting of AE1 to the apical rather than the normal location in the basolateral membrane of the α-intercalated cell (Fig 1).

It should be noted that the rate of H^+ secretion by α-intercalated cells is importantly influenced by the rate of Na^+ transport in the neighboring principal cells that are directly involved in Na^+ reabsorption and K^+ secretion but not in H^+ secretion. In the cortical collecting tubule, principal cells are the predominant type.[49-51] The lumen-negative potential difference usually prevailing in the cortical collecting tubule which is generated by active Na^+ reabsorption favors the secretion of H^+ and K^+ ions.[53-57] Maneuvers that obliterate lumen electronegativity in the cortical collecting tubule (CCT) have been shown to inhibit H^+ and K^+ secretion.[53-57] In contrast, the medullary collecting tubule secretes H^+ independently of Na^+ transport.[57-59] From these observations, we postulated that assessment of acidification by the CCT *in vivo* should be possible using maneuvers that enhance only sodium-dependent acidification.[23] Furosemide and other loop diuretics should provide a tool to assess sodium-dependent acidification by the CCT. We reasoned that this approach should be more practical than the administration of sodium sulfate, a powerful stimulus for sodium-dependent acidification.[23] First, by blocking NaCI reabsorption in the thick ascending Loop of Henle, loop diuretics increase Na^+ delivery to the collecting tubule. Second, by increasing Na^+ reabsorption in the CCT, they should result in the creation of a favorable transtubular voltage (lumen negative) and thus enhancement of H^+ and K^+ secretion. This postulation requires that a stimulatory effect of loop diuretics on CCT acidification and K^+ excretion be totally or partially prevented by amiloride, an agent well known to block Na^+ reabsorption and to thereby inhibit acidification and K^+ secretion in the CCT.[53] Third, furosemide does not exert any direct effect on acidification by the collecting tubule.[60] The notion that loop diuretics increase sodium-dependent acidification in the CCT was

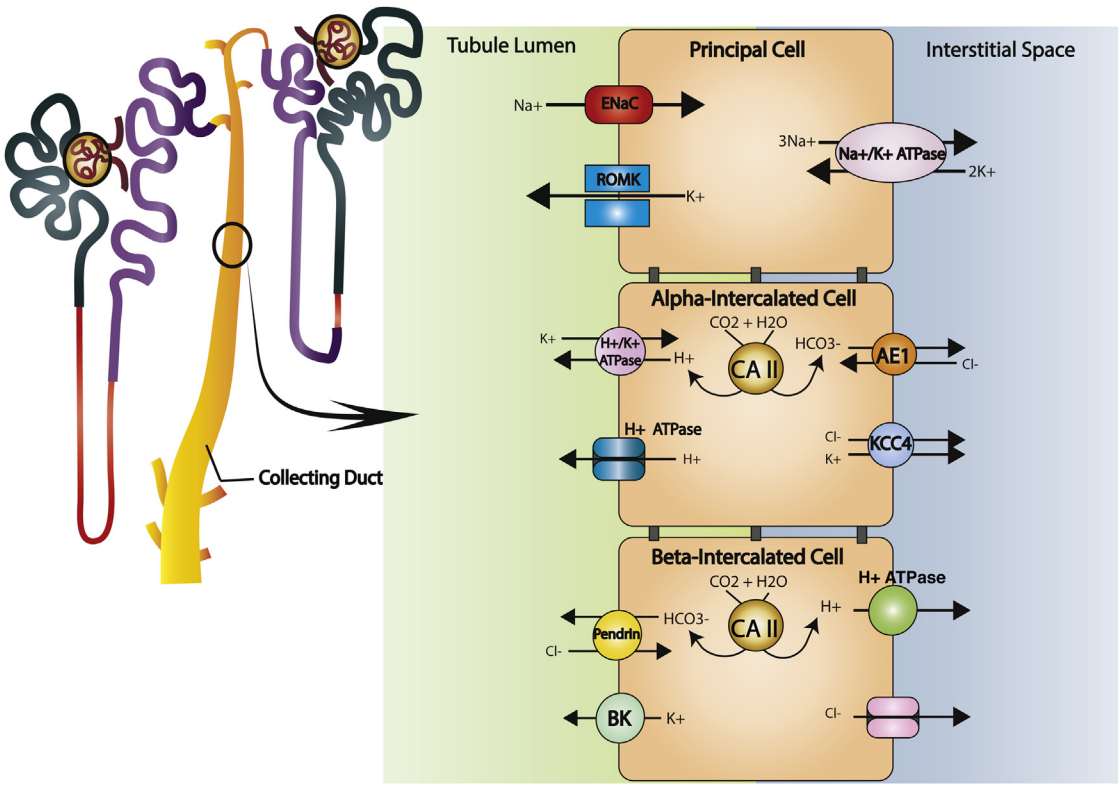

Figure 1. Transepithelial ion transport in principal and intercalated cells. The principal cell (upper panel), shown with the luminal membrane epithelial sodium channel, ENaC, and the renal outer medullary small-conductance K channel (ROMK).The type A intercalated cell (middle panel), bicarbonate and proton generation is catalyzed by cytosolic carbonic anhydrase II (CA II) providing protons for luminal V-type H^+-ATPases and bicarbonate for basolateral chloride/bicarbonate exchangers including AE1. Type A intercalated cells also express basolateral KCC4 KCl-cotransporters that may function in maintaining low intracellular chloride levels. Type A intercalated cells express also on their luminal membrane H^+/K^+-ATPases that serve mostly for preservation of potassium during potassium deprivation and hypokalemia. The type B intercalated cell (lower panel), these cells express a chloride/bicarbonate exchanger called pendrin on the luminal membrane, mediating bicarbonate secretion and chloride absorption. Bicarbonate is produced from CO_2 and H_2O catalyzed by CA II. Type B intercalated cells express a vacuolar-type H^+-ATPase in the basolateral membrane.

supported by showing that their stimulatory effect on urine pH and urinary acid excretion was prevented by amiloride (Fig 3).This was demonstrated both in normal subjects[23] and in patients with CKD, with and without and hyperkalemic DRTA.[21,61-63] This was also shown in patients with selective aldosterone deficiency and in adrenalectomized rats demonstrating that sodium-dependent acidification is, at least in part, independent of aldosterone.[61]

The obliteration of sodium-dependent acidification by amiloride reveals the importance of intact epithelial sodium channel (ENaC) activity for optimal distal urinary acidification. Distal delivery of sodium to the CCT is likewise very important for optimal H^+ secretion and thus for prevention/attenuation of metabolic acidosis. This was illustrated in a case study of a patient who self-induced metabolic acidosis with large doses of laxatives and was profoundly volume depleted with avid kidney retention of sodium.[64] In this patient, the correction of the volume deficit with daily infusions of sodium chloride increased distal sodium delivery and corrected the metabolic acidosis without the administration of alkali therapy[64] (Fig 4). It should be noted, however, that in DRTA, the price of attempting to increase sodium-dependent acidification in DRTA is an exaggerated increase in K^+ excretion (because H^+ secretion is impaired) and thus the attendant K^+ wastage with severe hypokalemia. The mechanisms of development of hypokalemia in classic RTA or DRTA are discussed later in this article.

Inherited or Primary DRTA

DRTA may occur as a primary inherited entity or may be acquired as a result of renal tubular damage due to a variety of renal diseases, systemic conditions, or drugs. In children, DRTA is most often observed as a primary or hereditary entity. Because these patients are usually treated with alkali therapy successfully, they experience normal development and are also seen as adults as well. At present, the known mutations resulting in inherited DRTA have been identified in transporters present in α-intercalated cells. These include mutations in the B1 and a4 subunits of H^+-ATPase, AE1, and cytosolic CA II.[33,38,43]

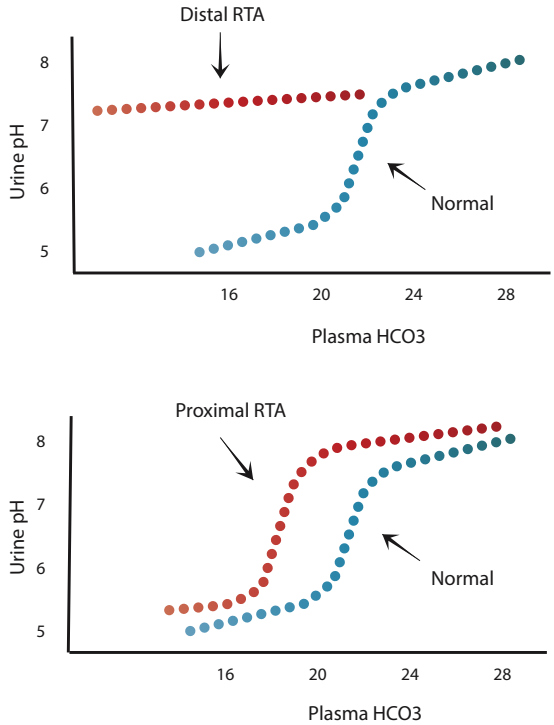

Figure 2. Differences in urine pH and plasma bicarbonate level in distal renal tubular acidosis (RTA) (upper panel) and proximal RTA (lower panel). Modified from Haque and colleagues.[44]

CA II Gene Mutations

By catalyzing the hydration of CO_2 to H^+ and HCO_3^-, cytosolic CA II plays a key role in the intracellular generation of these ions from CO_2 that enters the tubular cells (Fig 1). In fact, the first defect causing inherited DRTA was that attributable to congenital CA deficiency.[65] CAs are zinc metalloenzymes that catalyze the reversible hydration of CO_2 to form $HCO3^-$ and H^+. There are 15 known CAs of which CA II is the most widespread and has the highest catalytic activity.[66] CA II, CA IV, and CA XII are expressed by the human kidney.[66] In the kidney, CA II is present in the proximal tubule, thin descending limb, thick ascending limb, and intercalated cells of the cortical outer and inner medullary collecting tubule.[67]

Many different mutations causing RTA have been identified in several families. The pattern of inheritance is autosomal recessive.[68] Mutations in CA II lead to CA II deficiency, which is measurable in circulating erythrocytes.[69] CA II deficiency has been reported in several ethnic backgrounds, including Arabic, Italian, German, French, Hispanic, and African-American.[69] Consanguinity is a common feature in families with CA II mutations.[70-71]

More than 70% of the cases have been reported from the Arabian Peninsula.[70,71] A study done in patients from Tunisia and Algeria traced the ancestry of all affected patients studied to an old Arab tribe of Helal that had settled there in the 10th century. Clinically, Arabic patients have a very severe phenotype, and severe cognitive impairment is a consistent feature.[72] Some patients present with osteopetrosis, RTA, cerebral calcification, and developmental delay, but no cognitive impairment. CA II deficiency can cause recessive mixed proximal-distal (type III) RTA.[72] A predominance of the DRTA has been reported in some cases.[73-75]

An autosomal recessive syndrome of osteopetrosis, RTA, and cerebral calcification was initially reported in 1972.[76,77] The cause was not known until 1983 when CA II deficiency was identified as the main defect by Sly and colleagues.[65] Whyte and colleagues[73] investigated 3 sisters who had presented with osteopetrosis in infancy. Although osteopetrosis resolved spontaneously, during adolescence, 2 of them developed basal ganglia calcification. Later, RTA was diagnosed. Whyte and colleagues[73] suggested that acidosis might have led to spontaneous resolution of osteopetrosis. In 2004, Shah and colleagues[69] studied 20 families referred for CA II deficiency. All patients studied had osteopetrosis, RTA, and developmental delay. Cerebral calcification was present in most of the patients. Other studies have reported facial dysmorphism with low-set ears, hypertelorism, and a depressed nasal bridge.[78,79] There have been very few reports of nephrocalcinosis and kidney stones.[80] Mild conductive hearing loss has been reported from Saudi Arabia.[79,81] Middle-ear effusion and ossicular ankylosis as a result of osteopetrosis were found to be the cause.

V-ATPase Gene Mutations

V-ATPases are ubiquitous multisubunit complexes mediating ATP-driven vectorial transport of protons across membranes.[82,83] They can be fractionated into a V1 domain of 640 kDa and a membrane-associated V0 domain of 240 kDa.[83-85] The peripheral domain (V1) hydrolyzes ATP, and the integral domain (V0) conducts protons. Most of the subunits are expressed as multiple transcript variants from multiple genes with tissue- and cell type–specific expression patterns.[82] V-ATPases are expressed at very high density in the plasma membranes of several specialized cells in different organs, including the kidney, the inner ear, the epididymis, and bone.[84]

The subunits reported to be involved in human disease are B and a of the V1 and V0 domains, respectively. Subunit "a" has 4 isoforms (a1 to a4), and subunit B has 2 (B1 and B2).[84] The isoforms that are present in a limited number of tissues, such as those in the kidneys, are B1 (*ATP6V1B1*) and a4 (*ATP6V0A4*). In the kidney, V-ATPases are localized in the apical membrane of α-intercalated cells and in the basolateral membrane of β-intercalated cells[85] (Fig 1).

Mutations in *ATP6V1B1* and *ATP6V0A4* genes, encoding intercalated cell–specific B1 and a4 subunits, respectively, have been associated with early onset cases of DRTA which are always inherited in a recessive manner.[36,37,85] Many patients with DRTA and nerve deafness present with mutations in the *ATP6V1B1* gene encoding the B1 subunit of H^+-ATPase.[36] The *ATP6V1B1* gene is also expressed in interdental cells and endolymphatic sac epithelia, accounting for the hearing impairment associated with DRTA caused by mutations in this gene; the hearing loss was bilateral, asymmetrical, and progressive, occasionally with a conductive component.

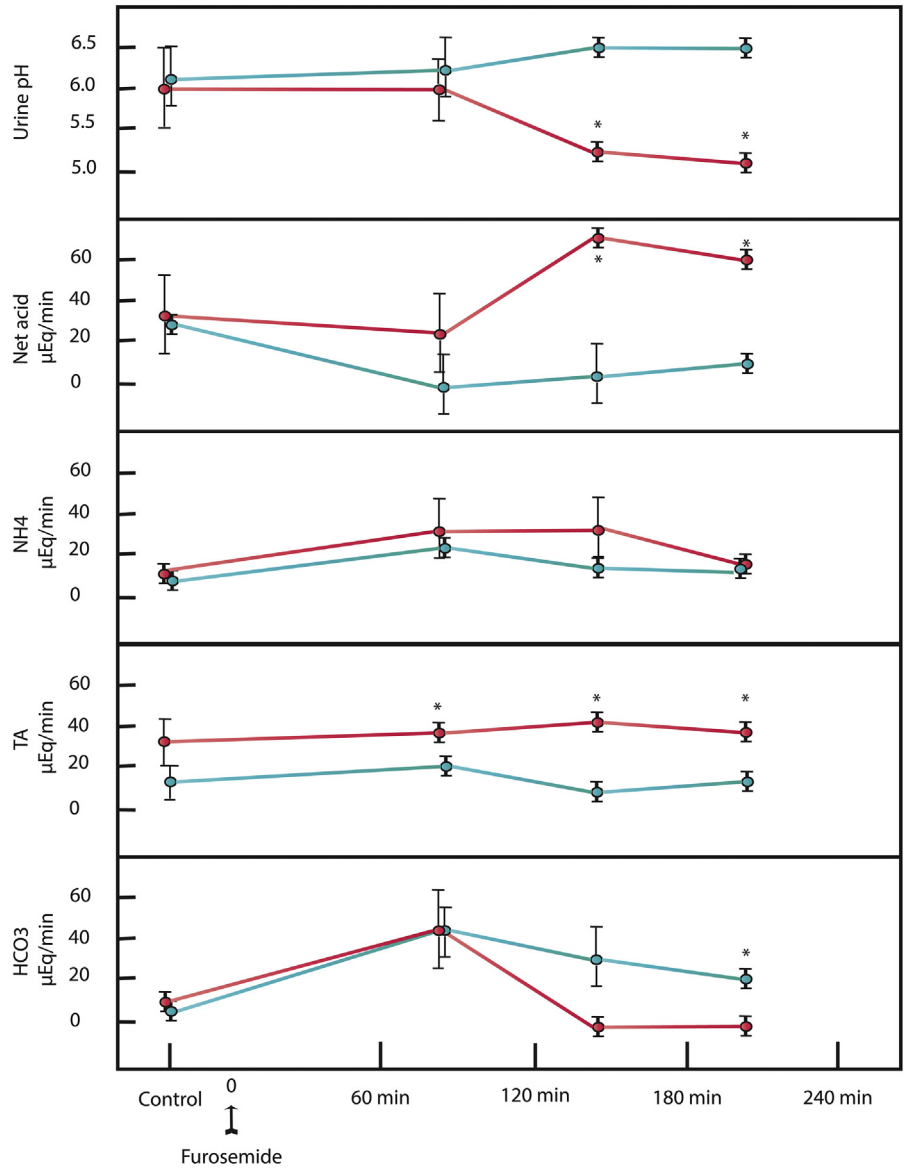

Figure 3. The effect of furosemide (red line) and furosemide + amiloride (blue line) on urinary acidification in normal subjects. The asterisk donates a significant difference between the 2 experimental conditions. Modified from Batlle, Elsevier, 1986.[23]

Sporadic or autosomal recessive DRTA without sensorineural deafness can be caused by mutations in the gene *ATP6V0A4* encoding the 116-kDa subunit of V-ATPase.[37] Thirteen kindred with normal hearing were studied. They had presented in early childhood with severe metabolic acidosis, hypokalemia, and normal renal function. Urinary pH was reported to be greater than 6.5. All had nephrocalcinosis, and with the exception of 2, all had elevated urine calcium. A follow-up of these patients found hearing loss in some at a later age. It is now known that *ATP6V0A4* is also expressed within the human inner ear. These findings therefore show that mutations in either *ATP6V1B1* or *ATP6V0A4* can cause hearing impairment.

The mechanisms whereby V-ATPase gene mutations result in impaired proton secretion are not completely understood. B1 and a4 subunit localization at the apical membrane of α-intercalated cells can be demonstrated by immunofluorescence.[84] *ATP6V1B1* and *ATP6V0A4* have been shown to display diminished pump activity despite normal pump assembly. Mutations were identified which produce highly disruptive changes likely to result in loss of function of the encoded *ATP6V1B*1 and *ATP6V0A4* proteins. Several mutations causing DRTA have been located to the gene encoding these subunits.[36,37,85] Premature termination codons and frameshift and splice-site mutations have been described in some, whereas in others, missense mutations were identified. The missense mutations are found in evolutionarily conserved residues predicted to interfere with subunit folding or with interaction with neighboring subunits during assembly

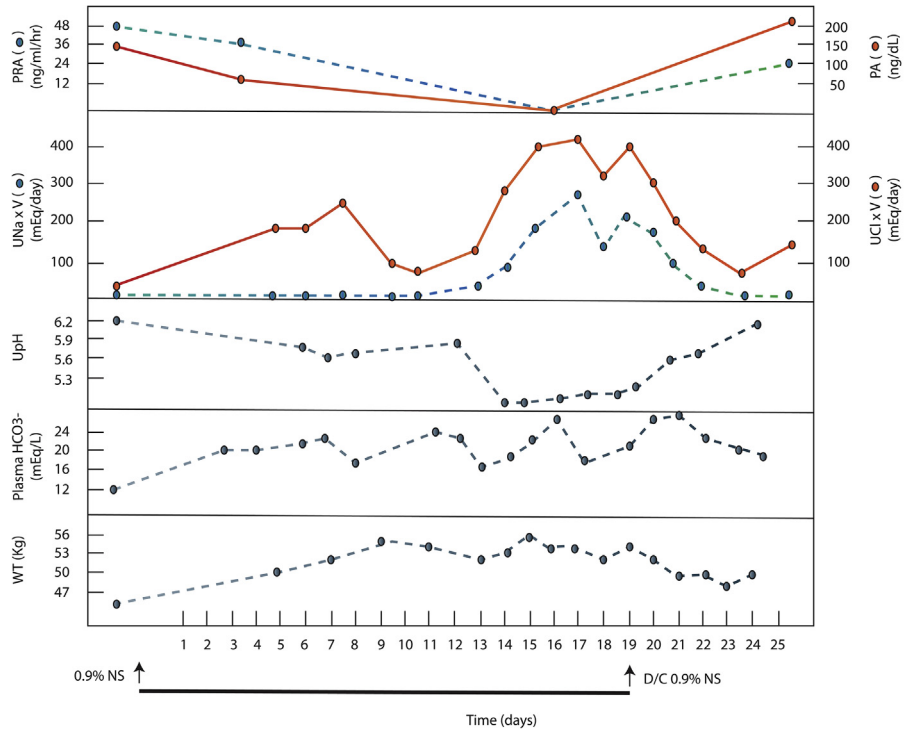

Figure 4. Changes in plasma renin activity (PRA), plasma aldosterone (PA), sodium excretion (UNa × V), chloride excretion (UCl × V), urinary pH (UpH), plasma bicarbonate, and body weight before the administration of 0.9 M solution of sodium chloride, during its administration (heavy bar), and after its discontinuation. Please note the increase in plasma bicarbonate during the administration of sodium chloride and the decline when it was discontinued. Adapted from Batlle.[64]

of the complex V-ATPase.[84] Yang and colleagues[86] showed that in response to acidification, mutants were not incorporated into enzymatically active partial V-ATPase complexes, preventing normal assembly, but trafficked to the apical membrane and inhibited microsomal proton pumping activity. In rat inner medullary collecting duct (IMCD) cells of GFP-B1 fusion proteins containing DRTA-associated missense mutations (from H^+-ATPase B1 and 31-kDa subunits).[86] In point mutations of the a4 subunit COOH terminus R807Q and G820R, Su and colleagues[87] found that G820R mutation caused a complete loss of phosphofructokinase-1 (PFK-1) binding to the a4 subunit without affecting PFK-1 activity, whereas R807Q mutation reduced a4 subunit production rendering V-ATPase inactive.[87] The G820R mutation in the a4 subunit was later shown to abrogate binding of the glycolytic enzyme PFK-1 and to reduce H^+-ATPase pump coupling without altering subunit abundance or targeting. Similar yeast expression of the DRTA-associated a4 mutant R807Q resulted in greatly reduced accumulation or loss and complete loss of activity.[87] Thus, in the context of DRTA, the stability and function of the metabolon composed of H^+-ATPase and glycolytic components can be compromised by either loss of required PFK-1 binding (G820R) or loss of pump protein (R807Q).[87]

AE1 Mutations

The transport of bicarbonate across the basolateral membrane back into the blood is effected in the α-intercalated cells by AE1, a polypeptide product of the *SLC4A1* gene (Fig 1). Primary DRTA may be observed sporadically or with autosomal dominant or recessive transmission.[33-35,41,75,88,89,90] Initially, only the dominant transmission was reported with AE1 mutations, but later, Karet and colleagues[88] found families with autosomal recessive disease. Clinical histories revealed differences between patients with dominant and recessive disease that may prove useful in the screening of future patients. Interestingly, dominant *AE1* mutation cases can present with either complete or incomplete DRTA.[34,90] In contrast, autosomal recessive patients always present with complete DRTA. Age at presentation was much younger in recessive patients, often diagnosed in infancy. Hypokalemia was more severe, and all recessive patients exhibited growth retardation. Nephrocalcinosis was found in all patients, even in a neonate. In contrast, the dominant patients were identified at an older age, and none of the affected members at 10 years of age had radiologic evidence of nephrocalcinosis. The autosomal recessive *AE1* mutations that have been reported are associated with hemolytic anemia as a result of Southeast Asia ovalocytosis.[89] Hemolytic anemia, however, is often not present in autosomal recessive *AE1* mutations.

The molecular mechanisms that render a mutated protein dysfunctional may involve an array of defects spanning from internal sequestration of a given transporter to its mistargeting to the plasma membrane (reviewed in

the study by Batlle and Haque).[43] Mutations of *AE1* are thought to give rise to DRTA through 2 types of dysfunction in biosynthetic targeting.[43] The first is intracellular retention with dominant negative inhibition of the polypeptide product of the coexpressed wild-type allele. Thus, the first detected *AE1* missense mutation associated with DRTA, AE1 R589H, is retained in the endoplasmic reticulum of Madin-Darby canine kidney (MDCK) cells and prevents its trafficking to the basolateral membrane.[91] A second class of dominant DRTA-associated AE1 mutant is delivered to the cell surface but with reversed polarity or loss of polarity. The dominant DRTA mutant AE1 901X, with a truncated C-terminal cytoplasmic tail, was localized either to the apical membrane[92] or to both apical and basolateral membranes of polarized MDCK cells[93] effectively short-circuiting acid secretion by cosecreting HCO_3^- and H^+ into the collecting duct lumen. The most common recessive DRTA-causing mutation, G701D, interacts with the chaperone glycophorin, which appears to rescue the mutant protein in red blood cells.[94] Glycophorin is absent from intercalated cells, possibly explaining the cell-specific phenotype of this particular mutation.[95] The autosomal recessive pattern of mutations such as G701D or S773P may be explained by the intracellular retention of mutant proteins while enough normal proteins reach the membrane in heterozygous patients.[91] Misfolding, which leads to impaired function and decreased half-life, has been described with S773P-recessive mutant proteins causing DRTA.[41]

CLINICAL FEATURES

In children, DRTA is most often observed as a primary entity.[38,43,45] Stunted growth and bone loss are often present because of chronic metabolic acidosis. Other associated features that lead to the initial diagnosis include nephrocalcinosis, nephrolithiasis, hypercalciuria, and hypocitraturia. As a consequence of metabolic acidosis, skeletal buffers such as carbonate and phosphate in combination with calcium are removed from the bones resulting not only in bone demineralization but also contributing to hypercalciuria. In addition, expression of renal calcium transport proteins is decreased in metabolic acidosis further promoting calcium excretion and thus development of nephrocalcinosis and kidney stones.[39] Metabolic acidosis induced by ammonium chloride causes resistance to the anticalciuric effect of parathyroid hormone.[96] This could also contribute to the hypercalciuria observed in chronic metabolic acidosis. Citrate excretion is reduced in primary DRTA but not in proximal RTA. Consistent with this difference in citrate excretion, kidney stones are frequent in distal RTA but rare in proximal RTA. Most of the citrate reabsorbed in the proximal tubule is coupled with sodium reabsorption via NaDC-1.[97] In DRTA, but not in proximal RTA, the metabolic acidosis appears to exert the normal stimulating effect on this transporter, thereby causing a decrease in citrate excretion which predisposes to nephrolithiasis, a key clinical manifestation of the disease.

Patients may suffer from weakness and muscle paralysis due to hypokalemia from renal potassium wastage. Hypokalemia is indeed a striking feature of distal, but not proximal, RTA.[98] Polyuria is often encountered and is related to the reduced capacity to concentrate urine most likely due to both hypokalemia and nephrocalcinosis. The polyuria can therefore be attributed to an acquired form of nephrogenic DI. Hypermagnesiuria, without changes of plasma Mg^{++}, is a characteristic finding in acidotic patients with primary DRTA and appears to be correctable by administration of sodium bicarbonate.[99]

Progression of nephrocalcinosis may lead to development of CKD.[45,100-103] Hypokalemic nephropathy associated with tubular hypertrophy and dilatation may cause renal medulla cyst formation.[104] In a study by Evan and colleagues, the renal histology of patients with primary DRTA revealed damage to inner medullary collecting tubules and Bellini ducts, epithelial cell damage, and calcium phosphate deposition.[105] Cyst formation has been documented in hypokalemic states, and it is possible that for the rare patients who progress to CKD, there may be a component of hypokalemic nephropathy that adds to nephrocalcinosis as the cause of declining kidney function. Therapeutic correction of the acidosis early in life by continuous alkali administration induces resumption of normal growth and slows down the progression of nephrocalcinosis with preservation of renal function.[13,100]

DRTA has been recently reported in 28 patients (range: 1-18 years), with molecular diagnosis in 19 patients of whom 6 had mutations in *ATP6V1B1*, 10 in *ATP6V0A4*, and 3 in SLC4A1.[101] Children with autosomal recessive DRTA presented at a median age of 3 months. A height of below −2 standard deviation score and nephrocalcinosis were present in all the patients. Medullary cysts were reported in 9 of 24 children.[101] Two-third of this cohort presented with partial renal Fanconi syndrome with tubular proteinuria, decreased tubular reabsorption of phosphate, and mild generalized aminoaciduria. After initiation of alkali treatment, the features of renal Fanconi syndrome disappeared in all patients, establishing the correct diagnosis of DRTA in some children who had previously been diagnosed with renal Fanconi syndrome of unknown etiology.

Extrarenal manifestations depend on the gene mutated and the type of mutation. Hemolytic anemia may be seen in some types of hereditary RTA associated with AE1 mutations, whereas sensorineural deafness is an important feature in H^+-ATPase mutations (reviewed in the study by Batlle and Haque).[43] In a recent study by Palazzo and colleagues,[45] next-generation sequencing was used in the evaluation of 89 patients with a clinical diagnosis of DRTA, to examine the prevalence of genetic defects in *SLC4A1*, *ATP6V0A4*, and *ATP6V1B1* genes and the clinical phenotype. A complete functional evaluation was not provided in this article, but the diagnosis of DRTA was supported by a positive urine anion gap and failure to lower the urine pH. A genetic cause was determined in 71.9% of cases. The mean age at diagnosis was significantly higher in patients with mutations in the *SLC4A1* gene than that of patients with mutations in the *ATP6V1B1* and *ATP6V0A4* genes. In addition, 92% were reported to have hearing impairment in the group of patients carrying causative mutations in the *ATP6V1B1*

gene and 56.7% of the patients with pathogenic variants in the *ATP6V0A4* gene. Nephrocalcinosis was found in 93% of the patients with mutations. Failure to thrive was reported as a common clinical sign at the onset of suspected DRTA in more than 70% of the patients with mutations in the *ATP6V1B1* and *ATP6V0A4* genes. Hypokalemia was present in 60% of the patients with *ATP6V1B1* and *ATP6V0A4* mutations.[45]

DRTA Variants

The term incomplete DRTA was introduced by Wrong and Davies[106] to designate patients who could not maximally lower the urine pH when challenged by a 1-day ammonium chloride loading test and yet were not spontaneously acidotic. Because these patients were not spontaneously acidotic, it was proposed that they had an acidification defect not yet clinically manifested, and the term incomplete DRTA was therefore coined. The 3 patients originally described by Wrong and Davies and others later described[107,108] did not appear to have decreased ammonium excretion, which explains the lack of associated metabolic acidosis. The clinical counterpart of this variant of DRTA would be a predisposition to kidney stones and a potential for conversion from incomplete to complete DRTA over time which, however, has been rarely reported. In fact, one could argue that incomplete DRTA is not a real entity but just a variant of normal capacity for urine acidification. Indeed, most studies in this area have lacked normal controls, and it remains unclear whether or not controls would have been able to acidify the urine by the criteria of the acute acid challenge where urine pH is expected to fall below 5.3. Because of the possible association between incomplete DRTA and kidney stone formation, there has been renewed interest on the topic of incomplete dRTA.[109-111] Of note, cases of incomplete DRTA have been reported as a result of screening for hypocitraturia in families with members suffering from complete DRTA.[102]In the clinical context of hypocitraturia and kidney stone formation potential, the screening for incomplete DRTA would make sense. This is not to say, however, that the ammonium chloride test would be the most sensitive test to detect defects in distal urinary acidification. Patients have been described with an inability to increase urine pCO_2 during bicarbonate loading who were able to lower urine pH below 5.3 during systemic acidosis.[112,113] All but one of the patients we reported with this pattern of distal acidification did not have spontaneous metabolic acidosis.[112,113] Many were treated with lithium and had therapeutic levels of it that they had not developed metabolic acidosis.[112] Therefore, the definition of incomplete DRTA could be expanded to include patients in whom the only detectable abnormality in urinary acidification is a failure to increase the urine pCO_2 normally, ie, above 60 mm Hg during $NaHCO_3$ loading. Normal volunteers increase urine pCO_2 above 80 mm Hg during bicarbonate loading.[112-114] Bicarbonate loading, however, is a time-consuming test to be performed properly and should be carried out in specialized clinics with careful attention to plasma sodium and osmolality as large amounts of sodium bicarbonate are required to alkalinize the urine to a pH of 7.8 or higher.

Another variant of RTA is the combination with features of proximal RTA. In these cases, there is a reduction in tubular reclamation of filtered HCO_3^- as well as an inability to acidify the urine maximally despite severe degrees of systemic acidemia. This pattern may be observed as a transient phenomenon in infants and young children with primary DRTA and does not represent a different genetic entity.[11] Combined proximal RTA and DRTA is also observed as the result of inherited carbonic anhydrase II deficiency.

Acquired DRTA

Numerous conditions have been associated with acquired DRTA that is often accompanied by hyperkalemia.[12-15] Among the causes of acquired DRTA associated with hypokalemia, the most common ones are autoimmune disorders.[111-128] Sjögren syndrome (SS) is an important cause of acquired DRTA and very rarely can be associated with proximal tubule defects.[125] In fact, SS is likely the most frequent cause of acquired hypokalemic DRTA. Moreover, incomplete RTA, defined by normal serum electrolytes but unable to lower urine pH maximally, is also common.[118,119] In the largest study to date of 130 patients with SS and renal involvement, 95 (73%) developed RTA, 91 with DRTA (66 complete and 25 incomplete) and 4 with PRTA and Fanconi syndrome. Nine patients presented with hypokalemic paralysis.[119] Bossini and colleagues[116] reported complete and incomplete DRTA in 60 patients with SS. The ammonium chloride (NH_4Cl) test, however, was only performed in 12 patients. Recently, urinary acidification was assessed in 57 SS patients using NH_4Cl and furosemide and fludrocortisone (FF).[118] The prevalence of complete DRTA was 3 in 57 patients (5%) but that of incomplete DRTA was 14 of 57 patients (25%). All subjects underwent both the urinary acidification tests using NH_4Cl and FF on separate days with a minimum of 1 week between the tests. Compared with NH_4Cl, the positive and negative predictive values of FF were 46% and 82%, respectively.[118]

Other manifestations of this chronic autoimmune disorder have been studied best in adult-onset SS.[126] Because they are so rare in pediatric population, most manifestations in pediatric patients have not been studied systematically, and diagnosis and management are usually inferred from experience with adults.[120] In a recent report, 12 pediatric cases were identified: 2 from chart review and 10 from the literature. RTA was mostly associated with primary SS. Parotid gland swelling, the most common presenting finding in pediatric SS, was documented in half the cases.[121] RTA was detected at the onset of SS or up to 9 years later. Impressively, hypokalemia was present in 92% of cases. A diffuse "tubulopathy" from interstitial nephritis was the predominant histopathological finding. RTA could be determined in 9 cases and was distal in 4 of 9 (44%) and proximal in 2 of 9 (22%), whereas 3 of 9 cases (33%) had features of both. SS should be considered in the older children with otherwise unexplained RTA.[121]

Kidney biopsies in patients with SS typically show features of chronic interstitial nephritis with focal or diffuse plasma lymphocytoid infiltration.[122] Although the pathogenesis of the RTA in SS is unclear, immune-mediated damage to acid-secreting cells in the kidney has been proposed. Several reports suggest that autoantibodies against carbonic anhydrase or acid-base transporters are involved in the pathogenesis of DRTA in SS. Cohen and colleagues[122] first showed lack of staining for H^+-ATPase in collecting tubules from patients with SS. Defranco and colleagues[123] used antibodies to the 31- and 56-kD kidney-specific subunits of H^+-ATPase, demonstrating complete absence of these vacuolar pumps. In addition, an antibody against the anion exchanger (AE1) present in the basolateral membrane of intercalated cells did not react with the patient's kidney. In another study of 46 patients with SS (13 with RTA), the patients with RTA had significantly elevated antibody levels to CA II.[124] The same authors reported that mice injected with human CA II developed antibodies against CA II and a syndrome pathologically similar to SS with lymphocyte and plasma cell infiltration of the salivary glands and kidneys. When challenged with an acid load, the animals were unable to acidify their urine.[127] It is unclear, however, if these findings are pathogenic or secondary to immune-mediated cellular damage.[128]

A similar histologic picture characterized by interstitial lymphocyte infiltration, hence an immunological cause for V-H^+ ATPase loss, has been suspected in kidney transplant recipients during acute or chronic graft rejection. Acquired DRTA has been well reported after kidney transplantation.[129-131] A study investigated the tubular expression of V-H^+ ATPase in kidney transplant recipients and found decreased staining in 50% of them.[132] However, the study was hampered by the low number of patients included and the use of a nonspecific V-H^+ ATPase antibody. Recently, in 14 transplant recipients who had DRTA type I, 5 patients were classified as having a rate-dependent RTA, 6 had type IV DRTA, and 12 had no RTA; immunostaining of the kidney was performed using a polyclonal antibody against the G3 subunit of V-H^+ ATPase.[133] The loss of V-H^+ ATPase expression was similar in patients with DRTA type I (21%), type IV (25%), and rate limited RTA (21%). The decrease of V-H^+ ATPase expression concerned mainly the apical location and not the intracellular distributed V-H^+ ATPase, which mediates vesicle acidification. Despite a decrease in V-H^+ ATPase expression in all transplant kidneys, only some of the recipients had clinical symptoms of DRTA, suggesting that they might have incomplete DRTA.[133] Alternatively, the decrease in staining may have been unrelated to any acidification defect.

It might be also possible that additional or separate defects in other proton-regulating pumps determine the extent of decreased acid excretion. A loss of AE1 and CA II in transplant recipients, however, has not yet been demonstrated in a large group.[133] In addition, related specific factors such as immunosuppressive therapy could be involved. Post-transplant DRTA has been related to cyclosporine A or tacrolimus.[134,135] Tanrisev and colleagues[136] evaluated adult renal transplant patients: 82 patients on mTORi and 55 patients on CNI. Frequency of RTA was 35% in the mTORi group and 42% in the CNI group. DRTA was common in both the groups and was affected by duration of time since transplantation and graft function.[136] The precise underlying mechanism of this effect of CsA is unknown. Cyclophilins A and B catalyze the isomerization of peptidyl prolyl bonds, thereby accelerating slow steps in protein folding and oligomerization.[137] The mechanism of conversion of β- to α-intercalated cell is critically dependent on the deposition of an extracellular matrix protein, hensin.[138] During the adaptation of β-intercalated cells to lowering the bath pH, hensin becomes deposited in the extracellular matrix (ECM) under the β cells. Deposition of hensin in the ECM requires extensive polymerization.[139] Productive formation of polymerized hensin from monomers with the underlying slow prolyl *cis-trans* isomerization reactions of the proline-rich segments of hensin might benefit from peptidyl prolyl *cis-trans* isomerase (PPIase) catalysis by avoiding aggregation-prone folding intermediates.[140] Watanabe and colleagues[141] suggested that hensin polymerization around adapting β-intercalated cells requires the PPIase activity of cyclophilins. CsA is able to prevent this adaptation by inhibition of a PPIase activity. This inhibitory effect of CsA may cause DRTA during acid loading.

The mechanism of development of an RTA picture in patients on mTOR inhibitors also remains unclear. V-H^+ ATPase function is necessary for activation of mechanistic target of rapamycin complex 1 (mTORC1). mTOR, which is a protein kinase, nucleates 2 complexes, mTOR complex 1 and complex 2 (mTORC1 and mTORC2). The role of the V-ATPase in mTORC1 signaling is unique in which the pump directly contributes to signaling of the pathway, although the precise mechanism of its involvement is unknown.[142] Recent studies have demonstrated that genetic deletion of *TSC1* (which encodes tuberous sclerosis complex protein 1), with ensuing constitutive activation of mTORC1 in collecting ducts, was associated with hyperkalemia and metabolic acidosis.[143] mTORC1 activation caused endoplasmic reticulum stress, columnar cell lesions, and dedifferentiation of collecting duct cells with loss of aquaporin-2 and epithelial-mesenchymal transition-like phenotypes.[143] Also, mTORC1 negatively regulated the expression of serum- and glucocorticoid-inducible kinase 1, a kinase crucial for collecting duct cell function, by regulating the expression and/or activity of ENaC, renal outer medullary potassium channel (ROMK), and Na^+/K^+-ATPase.[144] This could contribute to mTORC1 activation–induced aldosterone resistance and hyperkalemia.[143] However, for these results, it was not possible to delineate whether defective electrolyte handling was due to tubular dedifferentiation or primary changes in ion transport. Another recent *in vivo*–based study using structurally distinct competitive mTOR kinase inhibitors (PP242 and AZD8055) reported significant natriuresis but not kaliuresis.[145] Indeed, it was demonstrated that PP242 substantially inhibited Na^+ currents in isolated perfused CCDs but did not alter activity of K^+ inwardly rectifying channels (ROMK channels).[145] These new

findings suggest that mTOR, probably through mTORC2, preferentially regulates ENaC function rather than the associated K^+ secretory pathway ROMK.[145] In contrast, Grahammer and colleagues[146] showed that mTORC2 deficiency dramatically reduces ROMK activity, whereas ENaC activity was only mildly impaired. In transgenic mouse model of mTORC2 ablation from distal tubular segments, the authors showed a cell-intrinsic role of mTORC2 as a key regulator of K^+ handling. Increased K^+ levels resulted in an upregulation of mTORC2 expression and activity. mTORC2 in turn phosphorylates PKCα and serum- and glucocorticoid-inducible kinase 1, thereby regulating ROMK abundance and current at the plasma membrane. The clinical counterpart of these findings as it relates to Rapamycin use and its effect on plasma K^+ needs further work. It is well known however that Rapamycin use is associated with lower serum K^+ levels than the use of calcineurin inhibitors.[147]

Hypokalemia in DRTA: How Does It Develop?

The mechanism of the striking hypokalemia often seen in patients with DRTA has remained an enigma.[98] To date, the precise mechanism of hypokalemia remains unknown. A permeability defect, causing passive K^+ secretion, as it occurs with amphotericin B administration could readily explain it. Amphotericin B was first described as a cause of hypokalemic DRTA,[148] and the mechanism of defective acidification was soon uncovered by Steinmetz and Lawson using the bladder as an epithelial analog of the human collecting duct.[149] Altered ion permeability caused by amphotericin B explains some of its renal toxic effects.[150,151] Plasma renin activity and aldosterone do not increase during amphotericin B treatment.[152] All of the aforementioned details suggest that alterations in the permeability of tubular cells in the distal nephron are the likely cause of renal potassium wastage induced by amphotericin B. It is known that amphotericin B increases passive flux of cations, and this effect greatly augments K^+ secretion down its electrochemical gradient in the collecting tubule.[150] The impaired urine acidification associated with amphotericin B administration is caused by increased passive permeability of the luminal membrane and increased back diffusion of H^+, rather than by failure of active transport.[145,151] This ionic "leakiness" of the distal nephron for K^+ and H^+ induced by amphotericin B is the presumed cause of hypokalemic DRTA. Because a permeability defect as a mechanism of DRTA is unique to amphotericin B, however, alternative explanations are clearly required to explain the hypokalemia.[98] Exposure to toluene usually by inhalation (sniffing) also causes a picture of hyperchloremic metabolic acidosis with hypokalemia which resembles DRTA induced by amphotericin B.[153-156] Unlike amphotericin B, toluene even at very high concentrations does not cause proton back leak in turtle bladder preparations.[153] It should be noted that much of the metabolic acidosis that develops in toluene sniffers may be caused by organic acid overproduction from the metabolism of hippurate.[157] In fact, the plasma levels of hippuric acid in some cases can exceed 30 mg/mL and fall rapidly after cessation of sniffing; in this time, the metabolic acidosis resolves and plasma K^+ increases.[153] This is not to say, however, that these patients do not have a distal acidification defect. In a study in which we measured urine pCO_2 in alkaline urine after bicarbonate loading, a normal increase in urine pCO_2 was not seen in the 3 patients studied which is evidence for a decreased capacity for collecting tubule H^+ secretion.[153] Also consistent with DRTA is the finding that the urine pH is typically above 5.5 during metabolic acidosis, and moreover, the levels of urine ammonium were decreased in some of these patients.[153] In some cases, however, urine ammonium excretion is relatively high, which is not consistent with DRTA.[157] This may be because of transient DRTA in some cases of toluene sniffing or reflect that ammonium excretion is not sufficiently increased in the face of severe metabolic acidosis. In other words, were it not for the mild/reversible distal acidification defect, ammonium excretion would be higher in the face of severe metabolic acidosis. In a large study of toluene-intoxicated patients, most patients (18/20) showed some level of metabolic acidosis and hypokalemia, with evidence of renal K^+ wasting.[156] Mean urine pH was inappropriately high suggesting DRTA.[156] As to the cause of the urinary K^+ wastage, the excretion of hippurate mandates the excretion of a cation which may be NH_4^+, Na^+, or K^+.[157] Increased excretion of K^+ as a result of this may account in part for the transient hypokalemia seen in toluene sniffers.

The precise cause of hypokalemia in classic or type I RTA remains unknown. It is important to note that hypokalemia is a central feature of hereditary forms of DRTA in which the molecular mechanism is now known and attributable to specific acid-base transporters in α-intercalated cells which are not directly involved with K^+ transport (reviewed in the study by Moorthi and colleagues).[98] Therefore, the renal potassium wastage needs to be related most likely to increased K^+ secretion via ROMK in principal cells or via maxi-K voltage-gated potassium channel (BK) in β-intercalated cells or both as discussed in the following.

Sebastian and colleagues[158,159] proposed that patients with DRTA have some degree of sodium wastage with mild volume depletion leading to increased aldosterone levels and stimulation of collecting tubule K^+ secretion. Aldosterone over secretion could be expected as a result of sodium wastage, which has been documented occasionally in some patients with DRTA. It is unlikely, however, that aldosterone levels would be increased in the face of protracted potassium depletion, which suppresses aldosterone secretion, and indeed, few studies have reported aldosterone data from patients with severe hypokalemic DRTA. Factors other than secondary hyperaldosteronism need to be considered as responsible for the development of severe hypokalemia. Earlier studies had suggested that hypokalemia was ameliorated with sodium bicarbonate or phosphate but not sodium chloride.[160] Later, however, it was shown that hypokalemia was not corrected by treatment of the metabolic acidosis with alkali therapy.[159] This is not surprising considering that administration of bicarbonate

promotes K^+ secretion. Another contributing factor could be the polyuria and nephrocalcinosis that these patients develop. But that alone is not a satisfactory explanation.

An attractive (yet ruled out) mechanism that could account for hypokalemia is the existence of a defect in the renal K^+/H^+-ATPase pump.[161] Renal K^+ excretion is determined not only by active K^+ secretion localized largely to the distal tubule and cortical collecting tubule but also by active K^+ absorption localized, at least in part, to the outer medullary collecting tubule.[162,163] Activation of K^+ conservation in the outer medullary collecting tubule prevents further K^+ wastage while producing enhanced proton secretion. If the K^+/H^+-ATPase pump in the medullary collecting tubule was defective in DRTA, one would expect the development of both hypokalemia and metabolic acidosis. Thus, the classic features of RTA could be explained readily by such a defective mechanism. Vanadate, a nonspecific H^+-K^+-ATPase inhibitor, given to rats was reported to cause hypokalemia and metabolic acidosis, suggesting impaired H^+-K^+-ATPase activity as the mechanism responsible for hypokalemia and acidosis.[161] This explanation, however, cannot account for the finding that different genetic defects in acid-base transporters in intercalated cells are associated with hypokalemia as previously reviewed.[98] Moreover, no defect in genes encoding the H^+-K^+-ATPase pump has ever been reported in patients with DRTA. Simpson and Schwartz[164] described a case report of an infant with hypokalemic DRTA, the pathophysiology of which was proposed to be a deficiency or defect in the colonic H^+-K^+-ATPase activity. However, no genetic probes were available to confirm this proposition. In the absence of a direct genetic analysis, a role of the H^+-K^+-ATPase in causing DRTA, with severe hypokalemia, remains an unproven possibility. There could be a decreased K^+ reabsorption in the proximal tubule and the thick ascending limb causing K^+ wastage, but there is no evidence to support this mechanism either.

Could the acidosis itself play a role in the causation of hypokalemia? ENaC-mediated Na^+ transport in the cortical collecting tubule is increased by low pH,[165] and stimulation of ENaC activity can lead to enhanced K^+ secretion. Also a low intracellular pH inhibits basolateral Kir4.1/5.1 K^+ channel[166,167] which could decrease NCC activity in the distal tubule. This would increase cortical collecting tubule sodium delivery and increase ENaC activity. In patients with hypokalemic DRTA, the addition of amiloride may help reduce the amount of K supplementation which is consistent with preexisting increased ENaC activity in DRTA, but of course, there is no proof for it. What is known is that acidification and potassium secretion are clearly dependent on sodium transport in the collecting tubule and therefore inhibited by amiloride (Fig 3). It is possible that intactness of sodium transport or enhancement of it via ENaC stimulation by acidosis may cause augmented potassium secretion while H^+ secretion remains impaired by the very defect that causes the DRTA.

Hypokalemia development in DRTA could be conceptualized as the result of enhancement of potassium secretion attributable to an increase in voltage (lumen negative) in the collecting tubule. Normally, this negative voltage created by sodium transport in the cortical collecting tubule is attenuated by H^+ secretion. In the medullary collecting tubule, the voltage becomes lumen positive as a result of H^+ secretion. Failure to secrete hydrogen ions in DRTA can only result in a more favorable transtubular gradient for potassium secretion. According to this view, the administration of amiloride could correct the hypokalemia but could also aggravate the acidosis by diminishing H^+ secretion which is already curtailed by the very defect that causes DRTA. Although we favor this explanation, we realize that it may be over simplistic. An interesting study by Guetin and colleagues[168] provided new insights into the possible mechanisms of potassium wastage and salt wastage in DRTA. These authors used Finberg's mouse model of incomplete DRTA caused by disruption of the ATP6V1B1 gene encoding for the β1 subunit of the H^+-ATPase.[169] In this model, they found evidence of renal loss of Na^+, Cl^-, K^+, and water, causing hypovolemia, hypokalemia, and polyuria.[168] They interpreted their data as evidence that the cortical collecting tubule was the site of sodium wastage because the activity of both the apical ENaC and the apical pendrin/Na^+-driven chloride/bicarbonate exchanger was decreased.[168] ENaC activity in the medullary collecting tubule, by contrast, was markedly increased suggesting a localized inhibition in the cortex.[168] To explain this disparity, the authors proposed that β-intercalated cells, deficient on ATP6V1B1, impair the function of neighboring principal cells, which normally transport sodium, water, and potassium, through a paracrine ATP/prostaglandin E_2 (ATP/PGE_2) signaling cascade. PGE_2 production has a primary role in downregulating ENaC expression and function of the CCD of ATP6V1B1-deficient mice.[168] In the collecting tubule, luminal ATP via activation of purinergic receptors inhibits ENaC-dependent Na^+ transport.[170] Guetin and colleagues[168] concluded that β-intercalated cells are an important site of ATP release, which triggers the local production and release of PGE_2 through purinergic receptor activation, and that β-intercalated cells can downregulate ENaC in the neighboring principal cells through this paracrine system.

Of note, in this study, an increased abundance of the ROMK in the medulla and the BK channel in the cortex was found in mice with ATP6V1B1 deficiency.[168] These findings alone, in our view, help to explain the increased K^+ wastage in this model of incomplete DRTA and are likely relevant to human disease as well. The authors, however, proposed a complex mechanism whereby in this model of ATP6V1B1 deficiency in α-intercalated cells, the associated deficiency of H^+-ATPase in the β-intercalated cells induces release of PGE_2 through activation of calcium-coupled purinergic receptors.[168] Of interest also is the finding that indomethacin treatment for only 48 hours improved the abnormal ENaC regulation in the cortical collecting tubule suggesting a disturbance in PGE_2 paracrine signaling, whereas the reduction of pendrin is a direct consequence of proton-pump dysfunction. Apical ROMK channels are expressed in principal cells,

and K^+ secretion through ROMK channels depends on electrogenic Na^+ transport. It is unlikely that K^+ secretion occurs through excessive activity of ROMK in ATP6V1B1-deficient cortical collecting tubule because ENaC activity in this tubule segment is reduced. BK channels have been primarily detected in apical membrane of intercalated cells (Fig 1). High luminal flow rates are known to enhance K^+ secretion due to activation of BK channels.[171] Thus, elevated urinary flow in ATP6V1B1-deficient model probably activates BK channel and K^+ secretion. In line with this hypothesis, BK channel expression was increased in mutant mice, and a reduction of urinary flow by indomethacin normalized the expression level of BK channel and decreased urinary K^+ excretion in ATP6V1B1-deficient mice. Taken together, these results indicate that renal K^+ loss in ATP6V1B1-deficient mice may be in part a consequence of activation of flow-activated BK channels in β-intercalated cells. It would be very informative to examine the expression of BK and ROMK channel in kidney biopsies from patients with RTA.

Diagnostic Evaluation

The initial evaluation of someone suspected of having DRTA includes a clinical history for clues of associated renal and extrarenal manifestations of DRTA as well as family history for possibly afflicted members.[172] Extrarenal manifestations include the presence or absence of hearing impairment and stunted growth during childhood with episodes of hospitalization for electrolyte imbalances. A renal ultrasound to rule out or confirm nephrocalcinosis is always indicated.[172] Other clinical manifestations to look for are discussed under clinical features.

Laboratory investigations should include a 24-hour urine excretion analysis for the measurement of urinary calcium, citrate, and magnesium as well as sodium, potassium, and chloride. Random urine sample can provide information about the urine anion gap ($Na^+ + K^+ - Cl^-$) which is always positive despite the presence of metabolic acidosis in patients with DRTA.[173]

The urine anion gap is useful in the presence of metabolic acidosis as a way to estimate urine ammonium which is consistently reduced in DRTA but increased in other types of metabolic acidosis such as diarrhea.[173,174] Patients with DRTA always have a positive urine anion gap or very slightly negative, reflecting impaired ammonium excretion in the face of acidosis. When plasma bicarbonate is not reduced in the presence of CKD, the urine anion gap is predictably positive and should not be used as a marker for ammonium excretion. The significance and limitations of the urine anion gap have been recently discussed elsewhere.[174]

All that is usually needed for confirmation of DRTA is to document a low rate of acid excretion and failure to lower urine pH.[172] The urine pH is helpful to distinguish the different types of RTA. The urine pH is high in proximal RTA unless when plasma bicarbonate falls to a level in which the proximal tubule can reclaim the filtered bicarbonate load (Fig 2). Therefore, urine pH can be reduced almost normally when plasma bicarbonate is low, whereas it is never below 5.5 in patients with hypokalemic DRTA. The urine pH is also low in RTA associated with aldosterone deficiency or type IV RTA because of low buffer excretion.

Urine pH can be evaluated during spontaneous metabolic acidosis or after administration of an acidifying salt. It can also be assessed by the infusion of sodium sulfate or after giving a loop diuretic such as furosemide or bumetanide (Fig 3). These provocative tests assess Na^+-dependent acidification and can provide additional useful mechanistic information. Furosemide or bumetanide increase distal Na^+ delivery, thereby enhancing then-negative transepithelial potential in the collecting duct and stimulating H^+ and K^+ secretion. The mechanism of furosemide effect on urine pH can be shown by preventing sodium transport via the ENaC in the cortical collecting tubule using amiloride.[23] As shown in Figure 3, the lowering urine pH effect of furosemide is totally prevented by the concurrent administration of amiloride.[52] It is currently unknown to what extent the administration of amiloride would attenuate the effect of acid loading on urine pH and acid excretion. One would predict, however, that it would attenuate the stimulatory effect of acid loading on distal acidification to a significant extent given the importance of sodium-dependent distal acidification. To ensure sodium avidity, the furosemide test can be carried out with FF (1 mg) before administration of furosemide.[175]

When the urine is highly alkaline, as occurs after a properly conducted $NaHCO_3$-loading test, urine pCO_2 increases as a result of distal H^+ secretion. H^+ reacts with luminal HCO_3^- to form carbonic acid (H_2CO_3).[19-23] Carbonic acid dehydrates slowly in the medullary collecting duct to form CO_2, which is trapped in this area of the kidney. Provided that urine pH and HCO_3^-concentration increase above 7.8 and 80 mmol/L, respectively, the urine-to-blood pCO_2 gradient should be greater than 25 mm Hg in normal individuals. In patients with DRTA, the urine pCO_2 fails to increase, and the U–B pCO_2 gradient therefore remains only slightly above zero.[19-23,112-114] This is a test of capacity for H^+ secretion which is highly sensitive in detecting mild defects in distal acidification.[112,114]

Other Evaluations of Potential Interest

Immunohistochemical study of renal tissue using antibodies against H^+-ATPase and AE1 in renal biopsy specimens from DRTA patients is sparse but has provided important information on patients with congenital DRTA or certain forms of acquired DRTA by demonstrating that V-ATPase expression is very low or absent.[122,176-179] Moreover, the reduction of both H^+-ATPase and AE1 has been reported in intercalated cells from biopsies of DRTA patients. In acquired DRTA, some studies, however, have failed to demonstrate concordance between clinical chemistry data and immunohistochemistry.[177] Biopsy samples from patients with *AE1* mutation are rare, and only 2 cases have been reported. The absence of AE1 luminal expression was detected in the kidney from a patient carrying the S613F mutation.[176] However, some intracellular AE1 staining was detected; AE1 was absent from the basolateral side. Interestingly, the number of α-

intercalated cells appeared to be greatly reduced in this particular kidney biopsy, and the remaining α-intercalated cells appeared small and abnormal in shape. In the kidney from a patient with a R589H mutation and dominant DRTA, no AE1 staining was detected in intercalated cells that were reduced in number.[179] It would also be of great interest to examine the status of the potassium channels (ROMK and BK) and ENaC to gather information on mechanisms causing urinary potassium wastage and hypokalemia as described previously.

Recently, it has been reported that diagnosis of DRTA from B1 subunit mutations or nongenetic causes can potentially be confirmed by urinary exosome analysis.[46] True urinary exosomes are released into the urine by fusion of the outer membrane of the specialized multivesicular bodies with the apical plasma membrane during vesicular trafficking.[46,180] Pathare and colleagues[46] demonstrated that both B1 and B2 V-ATPase subunits can be specifically detected in human urinary exosomes by immunoblotting. It was shown that acute systemic acid-base changes exert rapid and significant changes in the abundance of the B1, but not the B2, subunit in human urine from healthy individuals, probably via a mechanism involving translocation of this apical protein. In contrast, in patients with inherited or acquired DRTA, the urinary B1 subunit was extremely low or undetectable and did not respond to acid loading in urine. No change in B2 subunit was found supporting a possible exclusive role of the B1 subunit for maximal distal urinary acidification in humans.[46]

The diagnosis of DRTA is essentially based on clinical and laboratory findings. However, genetic testing for *SLC4A1*, *ATP6V1B1*, and *ATP6V0A4* genes can be included as well. Next-generation sequencing had been reported to disclose new pathogenic variants in patients with a clinical diagnosis of primary RTA.[45,181]

TREATMENT

The aims of treatment of classic RTA are not only to correct the biochemical abnormalities but also to improve growth in children, maintain skeletal bone integrity, and prevent nephrolithiasis and nephrocalcinosis. This last point is important as progressive nephrocalcinosis ultimately may lead to CKD and occasionally to ESRD. A mixture of sodium and potassium salts is usually recommended. Polycitra (a mixture of sodium and potassium citrate) is an oral solution that contains 1 mEq of K^+ and 1 mEq of Na^+ per milliliter. The citrate, when metabolized by the liver, is equivalent to 2 mEq of HCO_3^- per milliliter. Alkali therapy should provide an adequate base to balance daily acid production.[182,183] Production of acid is higher in children (2 mmol/kg/d) than in adults (1 mmol/kg/d) because of H^+ release from bone during the process of skeletal growth. In young infants, as much as 5 to 8 mmol/kg/24 h of citrate (or bicarbonate) may be needed, whereas amounts of about 3 to 4 mmol/kg/24 h and 1 to 2 mmol/kg/24 h are required in children and adults, respectively. Potassium citrate alone can also be used, and in children, an amount of 4 mmol/kg/24 h is recommended. Citrate salts correct the hypocitraturia and prevent nephrolithiasis.[182] This organic anion also corrects the metabolic acidosis, thereby decreasing urine calcium excretion.

To normalize urine citrate, calcium, and also potassium excretion, patients are typically treated with K^+ citrate (1-4 mEq/kg/d) until the plasma bicarbonate increases to at least 22 mEq/L. With the administration of alkali usually in the form of citrate, the urine pH goes up which could foster kidney stone formation. This is usually offset by the benefit of reducing calcium excretion and increasing citrate excretion as the acidosis is corrected with alkali therapy. Thiazides have been considered to reduce calcium excretion and improve the acidosis, but they will worsen the hypokalemia and, in our opinion, should not be used.

Treatment of hypokalemia relies on the administration of potassium usually as potassium citrate. Occasionally, the use of amiloride has been reported to reduce potassium wastage.[184] Although we think that amiloride is effective to reduce K^+ wastage, studies in this area would be needed before using amiloride as part of the treatment of hypokalemic DRTA because of the concern that by reducing sodium-dependent acid excretion, it may worsen the metabolic acidosis. In cases in which the hypokalemia is very difficult to correct with large doses of potassium citrate, amiloride may be tried but with careful attention to the likely worsening of plasma bicarbonate level, which is to be anticipated.

Treatment of DRTA with alkali therapy, although effective, is sometimes challenging because of the "pill burden" as many tablets a day are needed to provide sufficient alkali dosing, and when liquid solutions are used, their poor palatability may reduce compliance.[185] Because of this, there may be a space for novel therapies for the treatment of metabolic acidosis. A prolonged-release granule combination product (ADV7103), with improved tolerability and palatability, is currently being tested. Another product for metabolic acidosis is TRC101, a sodium-free, nonabsorbed hydrochloric acid binder shown recently to increase serum bicarbonate in patients with CKD.[186] TRC101 is mechanistically distinct from bicarbonate supplementation as a means of correcting metabolic acidosis. TRC101 is an orally administered, insoluble, nonabsorbed polymer that selectively binds, retains, and removes H^+ and Cl^- under the pH and ionic conditions encountered in the human GI tract.[187,188] The high amine content of the polymer provides a substantial H^+-binding capacity (5-10 mEq/g polymer) sufficient to achieve the desired effect of serum bicarbonate normalization in a daily dose of the drug that is small enough to ensure good patient compliance (e.g., <10 g/d). The selective binding of Cl^- allows the unhindered GI absorption of larger anions from the intestine, such as short-chain fatty acids that serve as bicarbonate equivalents after they are metabolized.[187,188] Thus, serum bicarbonate could be increased with TRC101 treatment without the addition of exogenous bicarbonate (or as a way to reduce the relatively large supplementation usually needed). TRC101 is not an exchange resin and binds HCl without introducing a

counter ion; therefore, no changes in serum sodium, potassium, calcium, or magnesium are anticipated during TRC101 treatment. To our knowledge, this therapy has not been examined in patients with DRTA but may be worthy of consideration.

REFERENCES

1. Lightwood R. Communication No. 1. *Arch Dis Child.* 1935;10:205-206.
2. Butler AM, Wilson JL, Farber S. Dehydration and acidosis with calcification of the renal tubules. *J Pediatr.* 1936;8:489-499.
3. Pines KL, Mudge GH. Renal tubular acidosis with osteomalacia: report of three cases. *Am J Med.* 1951;11:302-311.
4. Albright F, Burnett CH, Parson W, Reifenstein EC Jr, Roos A. Osteomalacia and late rickets. The various etiologies met in the United States with emphasis on that resulting from a specific form of renal acidosis, the therapeutic indications for each etiological subgroup, and the relationship between osteomalacia and Milkman's syndrome. *Medicine.* 1946;25:399-479.
5. Latner AL, Burnard ED. Idiopathic hyperchloremic renal acidosis of infants. (Nephrocalcinosis infantorum.) Observations on the site and nature of the lesion. *Q J Med.* 1950;19:285-301.
6. Jackson WPU, Linder GC. Innate functional defects of the renal tubules, with particular reference to the Fanconi syndrome. Cases with retinitis pigmentosa. *Q J Med.* 1953;22:133-156.
7. Mudge GH. Clinical patterns of tubular dysfunction. *Am J Med.* 1958;24:785-804.
8. Huth EJ, Webster GD Jr, Elkinton JR. The renal excretion of hydrogen ion in renal tubular acidosis. III. An attempt to detect latent cases in a family; comments on nosology, genetics and etiology of the primary disease. *Am J Med.* 1960;29:586-598.
9. Soriano JR, Boichis H, Edelmann CM Jr. Bicarbonate reabsorption and hydrogen ion excretion in children with renal tubular acidosis. *J Peds.* 1967;71:802-813.
10. Morris RC Jr. Renal tubular acidosis. Mechanisms, classification and implications. *N Engl J Med.* 1969;281:1405-1413.
11. McSherry E, Sebastian A, Morris RC Jr. Renal tubular acidosis in infants: the several kinds, including bicarbonate-wasting, classic renal tubular acidosis. *J Clin Invest.* 1972;51:499-514.
12. Sebastian A, Schambelan M, Lindenfeld S, Morris RC Jr. Amelioration of metabolic acidosis with fludrocortisone therapy in hyporeninemic hypoaldosteronism. *N Engl J Med.* 1977;297:576-583.
13. Batlle DC, Arruda JA, Kurtzman NA. Hyperkalemic distal renal tubular acidosis associated with obstructive uropathy. *N Engl J Med.* 1981;304:373-380.
14. Batlle DC. Hyperkalemic hyperchloremic metabolic acidosis associated with selective aldosterone deficiency and distal renal tubular acidosis. *Semin Nephrol.* 1981;1:260-274.
15. Arruda JAL, Batlle D, Sehy JT, Roseman MK, Barabowski RS, Kurtszman NA. Hyperkalemia and renal insufficiency: a role of selective aldosterone deficiency and tubular defect. *Am J Nephrol.* 1981;1:160-167.
16. Thirakomen K, Kozlov N, Arruda JAL, Kurtzman NA. Hydrogen ion secretion following the release of unilateral ureteral obstruction. *Am J Physiol.* 1976;231:1233-1239.
17. Arruda JAL, Subbarayuda K, Dytko G, Mola R, Kurtzman NA. Voltage dependent distal acidification defect unduced by amiloride. *J Lab Clin Med.* 1980;95:407-416.
18. Seldin DW, Wilson JD. Renal tubular acidosis. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. *Metabolic Basic of Inherited Diseases.* 3rd ed. New York: McGraw-Hill; 1973:pp1548-1566.
19. Halperin ML, Goldstein MB, Halo A. Studies on the pathogenesis of type I (distal) renal tubular acidosis as revealed by the urinary pCO2 tensions. *J Clin Invest.* 1974;53:669-677.
20. Arruda JAL, Kurtzman NA. Mechanisms and classification of deranged distal urinary acidification. *Am J Physiol.* 1980;239:F515-F523.
21. Batlle D, Sehy JT, Roseman MK, Arruda JAL, Kurtzman NA. Clinical and pathophysiologic spectrum of acquired distal renal tubular acidosis. *Kidney Int.* 1981;20:389-396.
22. Batlle D, Kurtzman NA. Distal renal tubular acidosis: pathogenesis and classification. *Am J Kidney Dis.* 1982;1:328-344.
23. Batlle D. Segmental characterization of defects in collecting tubule acidification. *Kidney Int.* 1986;30(4):546-554.
24. DuBose TD Jr, Catflisch CR. Effect of selective aldosterone deficiency on acidification in nephron segments of the rat inner medulla. *Clin Invest.* 1988;82:1624-1632.
25. Steinmetz PR, Lawson LR. Defect in acidification induced invitro by amphotericin B. *J Clin Invest.* 1970;49:596-601.
26. Dixon TE, Al-Awqati Q. Urinary acidification in turtle bladder is due to a reversible proton-translocating ATPase. *Proc Natl Acad Sci U S A.* 1979;76:3135-3138.
27. Al-Awqati Q, Meuller A, Steinmetz PR. Transport of H+ against electrochemical gradients in turtle urinary bladder. *Am J Physiol Rev.* 1974;54:890-956.
28. Schambelan M, Sebastian A, Biglieri EG. Prevalence, pathogenesis, and functional significance of aldosterone deficiency in hyperkalemic patients with chronic renal insufficiency. *Kidney Int.* 1980;17:89-101.
29. Uribarri J, Oh MS, Pak CY. Renal stone risk factors in patients with type IV renal tubular acidosis. *Am J Kidney Dis.* 1994;23(6):784-787.
30. Batlle D, Flores G. Underlying defects in distal renal tubular acidosis: new understandings. *Am J Kidney Dis.* 1996;27(6):896-915.
31. Rodriguez Soriano J. Renal tubular acidosis: the clinical entity. *J Am Soc Nephrol.* 2002;13:2160-2170.
32. Sly WS, Whyte MP, Sundaram V, et al. Carbonic anhydrase II deficiency in 12 families with the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. *N Engl J Med.* 1985;313:139-145.
33. Alper SL. Genetic diseases of acid-base transporters. *Annu Rev Physiol.* 2002;64:899-923.
34. Bruce LJ, Cope DL, Jones GK, et al. Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (band 3, AE1) gene. *J Clin Invest.* 1997;100:1693-1707.
35. Tanphaichitr VS, Sumboonnanonda A, Ideguchi H, et al. Novel AE1 mutations in recessive distal renal tubular acidosis. Loss-of-function is rescued by glycophorin A. *J Clin Invest.* 1998;102:2173-2179.
36. Karet FE, Finberg KE, Nelson RD, et al. Mutations in the gene encoding B1 subunit of H^+-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet.* 1999;21:84-90.
37. Karet FE, Finberg KE, Nayir A, et al. Localization of a gene for autosomal recessive distal renal tubular acidosis with normal hearing (rdRTA2) to 7q33-34. *Am J Hum Genet.* 1999;65:1656-1665.
38. Fry AC, Karet FE. Inherited renal acidoses. *Physiology (Bethesda).* 2007;22:202-211.
39. Wagner K, Renkema KY, Velic A, et al. The calcium-sensing receptor promotes urinary acidification to prevent nephrolithiasis. *J Am Soc Nephrol.* 2009;20(8):1705-1713.
40. Devonald MA, Smith AN, Poon JP, et al. Non-polarized targeting of AE1 causes autosomal dominant distal renal tubular acidosis. *Nat Genet.* 2003;33:125-127.
41. Kittanakom S, Cordat E, Akkarapatumwong V, et al. Trafficking defects of a novel autosomal recessive distal renal tubular acidosis mutant (S773P) of the human kidney anion exchanger (kAE1). *J Biol Chem.* 2004;279:40960-40971.
42. Vargas-Poussou R, Houillier P, Le PN, et al. Genetic investigation of autosomal recessive distal renal tubular acidosis: evidence for early sensorineural hearing loss associated with mutations in the ATP6V0A4 gene. *J Am Soc Nephrol.* 2006;17:1437-1443.

43. Batlle D, Haque SK. Genetic causes and mechanisms of distal renal tubular acidosis. *Nephrol Dial Transplant.* 2012;27:3691-3704.
44. Haque SK, Ariceta G, Batlle D. Proximal renal tubular acidosis: a not so rare disorder of multiple etiologies. *Nephrol Dial Transplant.* 2012;27:4273-4287.
45. Palazzo V, Provenzano A, Becherucci F, et al. The genetic and clinical spectrum of a large cohort of patients with distal renal tubular acidosis. *Kidney Int.* 2017;91:1243-1255.
46. Pathare G, Dhayat NA, Mohebbi N, et al. Changes in V-ATPase subunits of human urinary exosomes reflect the renal response to acute acid/alkali loading and the defects in distal renal tubular acidosis. *Kidney Int.* 2018;93(4):871-880.
47. Batlle D, Ghanekar H, Jain S, Mitra A. Hereditary distal renal tubular acidosis: new understandings. *Annu Rev Med.* 2001;52:471-484.
48. Alper SL, Darman RB, Chernova MN, et al. The AE gene family of Cl/$HCO3^-$ exchangers. *J Nephrol.* 2002;15(Suppl 5):S41-S53.
49. Wagner S, Vogel R, Lietzke R, et al. Immunochemical characterization of a band 3-like anion exchanger in collecting duct of human kidney. *Am J Physiol.* 1987;253:F213-F221.
50. Schuster VL. Function and regulation of collecting duct intercalated cells. *Annu Rev Physiol.* 1993;55:267-288.
51. Alper SL, Natale J, Gluck S, et al. Subtypes of intercalated cells in rat kidney collecting duct defined by antibodies against erythroid band 3 and renal vacuolar H^+-ATPase. *Proc Natl Acad Sci U S A.* 1989;86:5429-5433.
52. Kurtz I, Dass PD, Cramer S. The importance of renal ammonia metabolism to whole body acid-base balance: a reanalysis of the pathophysiology of renal tubular acidosis. *Miner Electrolyte Metab.* 1990;16(5):331-340.
53. Stoner L, Burg M, Orloff J. Ion transport in cortical collecting tubule. Effect of amiloride. *Am J Physiol.* 1974;227:453-459.
54. Stokes JB, Ingram MJ, Williams AD, Ingram D. Heterogeneity of the rabbit collecting tubule: localization of mineralocorticoid hormone action to the cortical portion. *Kidney Int.* 1981;20:340-347.
55. Stokes JB. Potassium secretion by the cortical collecting tubule: relation to sodium absorption, luminal sodium concentration, and transepithelial voltage. *Am J Physiol.* 1981;241:F395-F402.
56. Koeppen BM, Helman STI. Acidification of luminal fluid by the rabbit cortical collecting tubule perfused in vitro. *Am J Physiol.* 1982;242:F521-F531.
57. Laski ME, Kurtzman NA. Characterization of acidification in the cortical and medullary collecting tubule of the rabbit. *J Clin Invest.* 1983;72:2050-2059.
58. Stone KD, Seldin DW, Kokko JP, Jacobson BR. Mineralocorticoid modulation of rabbit inedullary collecting duct acidification: a sodium—independent effect. *J Clin Invest.* 1983;72:77-83.
59. Lombard WE, Kokko JP, Jacobson BR. Bicarbonate transport in cortical and outer medullary collecting tubules. *Am J Physiol.* 1983;244:F289-F296.
60. Burg M, Stoner L, Cardinal J, Green N. Furosemide effect on isolated perfused tubules. *Am J Physiol.* 1973;225:119-124.
61. Batlle DC. Sodium dependent urinary acidification in patients with aldosterone deficiency and adrenalectomized rats. *Metabolism.* 1986;35:850-860.
62. Schlueter W, Keilani T, Hizon M, Kaplan B, Batlle DC. On the mechanism of impaired distal acidification in hyperkalemic renal tubular acidosis-evaluation with amiloride and bumetanide. *J Am Soc Nephrol.* 1992;3:953-964.
63. Rastogi S, Bayliss JM, Nascimento L, Arruda JA. Hyperkalemic renal tubular acidosis: of furosemide in patients and rats. *Kidney Int.* 1985;28:801-807.
64. Batlle DC, von Riotte A, Schuelter W. Urinary sodium in the evaluation of hyperchloremic metabolic acidosis. *N Engl J Med.* 1987;316(3):140-144.
65. Sly WS, Hewett-Emmett D, Whyte MP, et al. Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteoporosis with renal tubular acidosis and cerebral calcification. *Proc Natl Acad Sci U S A.* 1983;80:2752-2756.
66. Schwartz GJ. Physiology and molecular biology of renal carbonic anhydrase. *J Nephrol.* 2002;15(Suppl 5):S61-S74.
67. Purkerson JM, Schwartz GJ. The role of carbonic anhydrases in renal physiology. *Kidney Int.* 2007;71:103-115.
68. Sly WS, Sato S, Zhu XL. Evaluation of carbonic anhydrase isoenzymes in disorders involving osteopetrosis and/or renal tubular acidosis. *Clin Biochem.* 1991;24:311-318.
69. Shah GN, Bonapace G, Hu PY, et al. Carbonic anhydrase II deficiency syndrome (osteopetrosis with renal tubular acidosis and brain calcification): novel mutations in CA2 identified by direct sequencing expand the opportunity for genotype-phenotype correlation. *Hum Mutat.* 2004;24:272.
70. Fathaullah DM, Bejaoui M, Lepaslier D, et al. Carbonic anhydrase II(CA II) deficiency in Maghrebian patients: evidence for founder effect and genomic recombination at the CA II locus. *Hum Genet.* 1997;99:634-637.
71. Hu PY, Roth DE, Skaggs LA, et al. A splice junction mutation in intron 2 of the carbonic anhydrase II gene of osteopetrosis patients from Arab countries. *Hum Mutat.* 1992;1:288-292.
72. Bolt RJ, Wennink JM, Verbeke JI, et al. Carbonic anhydrase type II deficiency. *Am J Kidney Dis.* 2005;46. A50, e71-e73.
73. Whyte MP, Murphy WA, Fallon MD, et al. Osteopetrosis, renal tubular acidosis and basal ganglia calcification in three sisters. *Am J Med.* 1980;69:64-74.
74. Strisciuglio P, Hu PY, Lim EJ, et al. Clinical and molecular heterogeneity in carbonic anhydrase II deficiency and prenatal diagnosis in an Italian family. *J Pediatr.* 1998;132:717-720.
75. Alper SL. Familial renal tubular acidosis. *J Nephrol.* 2010;23(Suppl 16):S57-S76.
76. Guibaud P, Larbre F, Freycon MT, et al. Osteopetrosis and renal tubular acidosis. 2 cases of this association in a sibship. *Arch Fr Pediatr.* 1972;29:269-286.
77. Vainsel M, Fondu P, Cadranel S, et al. Osteopetrosis associated with proximal and distal tubular acidosis. *Acta Pediatr Scand.* 1972;61:429-434.
78. Borthwick KJ, Kandemir N, Topaloglu R, et al. A phenocopy of CAII deficiency: a novel genetic explanation for inherited infantile osteopetrosis with distal renal tubular acidosis. *J Med Genet.* 2003;40:115-121.
79. Muzalef A, Alshehri M, Al-Abidi A, et al. Marble brain disease in two Saudi Arabian siblings. *Ann Trop Paediatr.* 2005;25:213-218.
80. Ismail EA, Abul Saad S, Sabry MA. Nephrocalcinosis and urolithiasis in carbonic anhydrase II deficiency syndrome. *Eur J Pediatr.* 1997;156:957-962.
81. Zakzouk SM, Sobki SH, Mansour F, et al. Hearing impairment in association with distal renal tubular acidosis among Saudi children. *J Laryngol Otol.* 1995;109:930-934.
82. Vallés PG, Batlle D. Kidney vacuolar H^+-ATPase regulation. In: Nakamura S, ed. *Hand book of H^+ ATPases.* Pan Stanford Publishing; 2014:202-235.
83. Toei M, Saum R, Forgac M. Regulation and isoform function of the V-ATPases. *Biochemistry.* 2010;49:4715-4723.
84. Nishi T, Forgac M. The vacuolar (H+)-ATPases-nature's most versatile proton pumps. *Nat Rev Mol Cell Biol.* 2002;3:94-103.
85. Wagner CA, Finberg KE, Breton S, et al. Renal vacuolar H^+-ATPase. *Physiol Rev.* 2004;84:1263-1314.
86. Yang Q, Li G, Singh SK, et al. Vacuolar H^+-ATPase B1 subunit mutations that cause inherited distal renal tubular acidosis affect proton pump assembly and trafficking in inner medullary collecting duct cells. *J Am Soc Nephrol.* 2006;17:1858-1866.
87. Su Y, Blake-Palmer KG, Sorrell S, et al. Human H+-ATPase a4 subunit mutations causing renal tubular acidosis reveal a role for

interaction with phosphofructokinase-1. *Am J Physiol Renal Physiol.* 2008;295:F950-F958.
88. Karet FE, Gainza FJ, Gyory AZ, et al. Mutations in the chloride-bicarbonate exchanger gene AE1 cause autosomal dominant but not autosomal recessive distal renal tubular acidosis. *Proc Natl Acad Sci U S A.* 1998;95:6337-6342.
89. Bruce LJ, Wrong O, Toye AM, et al. Band 3 mutations, renal tubular acidosis and South-East Asian ovalocytosis in Malaysia and Papua New Guinea: loss of up to 95% band 3 transport in red cells. *Biochem J.* 2000;350(Pt 1):41-51.
90. Cheidde L, Vieira TC, Lima PR, et al. A novel mutation in the anion exchanger 1 gene is associated with familial distal renal tubular acidosis and nephrocalcinosis. *Pediatrics.* 2003;112:1361-1367.
91. Cordat E, Kittanakom S, Yenchitsomanus PT, et al. Dominant and recessive distal renal tubular acidosis mutations of kidney anion exchanger 1 induce distinct trafficking defects in MDCK cells. *Traffic.* 2006;7:117-128.
92. Toye AM, Banting G, Tanner MJ. Regions of human kidney anion exchanger 1 (kAE1) required for basolateral targeting of kAE1 in polarised kidney cells: mis-targeting explains dominant renal tubular acidosis(dRTA). *J Cell Sci.* 2004;117:1399-1410.
93. Rungroj N, Devonald MA, Cuthbert AW, et al. A novel missense mutation in AE1 causing autosomal dominant distal renal tubular acidosis retains normal transport function but is mistargeted in polarised epithelial cells. *J Biol Chem.* 2004;279:13833-13838.
94. Toye AM, Williamson RC, Khanfar M, et al. Band 3 Courcouronnes (Ser667Phe): a trafficking mutant differentially rescued by wild-type band 3 and glycophorin A. *Blood.* 2008;111:5380-5389.
95. Williamson RC, Toye AM. Glycophorin a: band 3 aid. *Blood Cells Mol Dis.* 2008;41:35-43.
96. Batlle DC, Itsarayoungyuen K, Hays S, Arruda JAL, Kurtzman NA. Parathyroid hormone is not anticalciuric during chronic metabolic acidosis. *Kidney Int.* 1982;22:264-271.
97. Hering-Smith KS, Mao W, Schiro FR, Coleman-Barnett J, Pajor AM, Hamm LL. Localization of the calcium-regulated citrate transport process in proximal tubule cells. *Urolith.* 2014;42:209-219.
98. Moorthi KM, Schlueter W, Kurtzman N, Batlle D. Distal renal tubular acidosis and the potassium enigma. *Semin Nephrol.* 2006;26:471-478.
99. Ariceta G, Vallo A, Rodriguez Soriano J. Acidosis increases magnesiuria in children with distal renal tubular acidosis. *Pediatr Nephrol.* 2004;19:1367-1370.
100. Rodriguez Soriano J, Vallo A, Castillo G, Oliveros R. Natural history of primary distal renal tubular acidosis treated since infancy. *J Pediatr.* 1982;101:669-676.
101. Caldas A, Broyewr M, Dechaux M, Kleinknecht C. Primary distal tubular acidosis in childhood: clinical study and long-term follow-up of 28 patients. *J Pediatr.* 1992;121:233-241.
102. Norman ME, Feldman NI, Cohn RM, Roth KS, McCurdy DK. Urinary citrate excretion in the diagnosis of renal tubular acidosis. *J Pediatr.* 1978;92:294-400.
103. Besouw MTP, Bienias M, Walsh P, et al. Clinical and molecular aspects of distal renal tubular acidosis in children. *Pediatr Nephrol.* 2017;32(6):987-996.
104. Igarashi T, Shibuya K, Kamoshita S, et al. Renal cyst formation as a complication of primary distal renal tubular acidosis. *Nephron.* 1991;59:75-79.
105. Evan AP, Lingeman J, Coe F, et al. Renal histopathology of stone-forming patients with distal renal tubular acidosis. *Kidney Int.* 2007;71:795-801.
106. Wrong O, Davies HE. The excretion of acid in renal disease. *Q J Med.* 1959;28:259-313.
107. Buckalew VM Jr, McCurdy DK, Ludwig GD, Chaykin LB, Elkinton JR. Incomplete renal tubular acidosis. Physiologic studies in three patients with a defect in lowering urine pH. *Am J Med.* 1968;45:32-42.
108. Tannen RL, Falls WF Jr, Brackett NC Jr. Incomplete renal tubular acidosis: some clinical and physiological features. *Nephron.* 1975;15:111-123.
109. Cameron MA, Maalouf NM, Adams-Huet B, Moe OW, Sakhaee K. Urine composition in type 2 diabetes: predisposition to uric acid nephrolithiasis. *J Am Soc Nephrol.* 2006;17:1422-1428.
110. Maalouf NM, Cameron MA, Moe OW, Sakhaee K. Novel insights into the pathogenesis of uric acid nephrolithiasis. *Curr Opin Nephrol Hypertens.* 2004;13:181-189.
111. Evan AP, Lingeman J, Coe F, et al. Worcester ERenal histopathology of stone-forming patients with distal renal tubular acidosis. *Kidney Int.* 2007;71(8):795-801.
112. Batlle D, Gaviria M, Grupp M, Arruda JAL, Wynn J, Kurtzman NA. Distal nephron function in patients receiving chronic lithium therapy. *Kidney Int.* 1982;21:477-485.
113. Batlle D, Grupp M, Gaviria M, Kurtzman NA. Distal renal tubular acidosis with intact ability to lower urine pH. *Am J Med.* 1982;72:751-758.
114. Batlle D. Delta pCo2 rather than urine-blood (U-B) pCo2 as an index of distal acidification. *Semin Nephrol.* 1982;2:189-191.
115. Wrong OM, Feest TG, Maciver AG. Immune-related potassium losing interstitial nephritis: a comparison with distal renal tubular acidosis. *Quart J Med.* 1993;86:513-534.
116. Bossini N, Savoldi S, Franceschini F, et al. Clinical and morphological features of kidney involvement in primary Sjögren's syndrome. *Nephrol Dial Transplant.* 2001;16:2328-2336.
117. Caruana RJ, Barish CF, Buckalew VM Jr. Complete distal renal tubular acidosis in systemic lupus: clinical and laboratory findings. *Am J Kidney Dis.* 1985;6:59-63.
118. Both T, Hoorn EJ, Zietse R, et al. Prevalence of distal renal tubular acidosis in primary Sjögren's syndrome. *Rheumatology (Oxford.* 2015;54(5):933-939.
119. Ren H, Wang WM, Chen XN, et al. Renal involvement and follow up of 130 patients with primary Sjögren's syndrome. *J Rheumatol.* 2008;35:278-284.
120. Cimaz R, Casadei A, Rose C, et al. Primary Sjögren syndrome in the paediatric age: a multicentre survey. *Eur J Pediatr.* 2003;162:661-665.
121. Pessler F, Emery H, Dai L, et al. The spectrum of renal tubular acidosis in paediatric Sjögren syndrome. *Rheumatology (Oxford).* 2006;45(1):85-91.
122. Cohen EP, Bastani B, Cohen MR, et al. Absence of H^+- ATPase in cortical collecting tubules of a patient with Sjogren's syndrome and distal renal tubular acidosis. *J Am Soc Nephrol.* 1992;3:264-271.
123. Defranco PE, Haragsim L, Schmitz PG, Bastani B. Absence of vacuolar H^+-ATPase pump in the collecting duct of a patient with hypokalemic distal renal tubular acidosis and Sjögren's syndrome. *J Am Soc Nephrol.* 1995;6:295-301.
124. Takemoto F, Hoshino J, Sawa N, et al. Autoantibodies against carbonic anhydrase II are increased in renal tubular acidosis associated with Sjögren's syndrome. *Am J Med.* 2005;118:181-184.
125. Wang C-C, Shiang J-C, Huang W-T, Lin S-H. Hypokalemic paralysis as primary presentation of Fanconi syndrome associated with Sjögren's syndrome. *J Clin Rheumatol.* 2010;16:178-189.
126. Brito-Zeron P, Ramos-Casals M, EULAR-SS task force group. Advances in the understanding and treatment of systemic complications in Sjögren's syndrome. *Curr Opin Rheumatol.* 2014;26(5):520-527.
127. Takemoto F, Katori H, Sawa N, et al. Induction of anti-carbonicanhydrase-II antibody causes renal tubular acidosis in a mouse model of Sjögren's syndrome. *Nephron Physiol.* 2007;106:63-68.
128. Rastegar A. Attending rounds: patient with hypokalemia and metabolic acidosis. *Clin J Am Soc Nephrol.* 2011;6:2516-2521.
129. Batlle D, Mozes MF, Manaligod J, Arruda JAL, Kurtzman NA. The pathogenesis of hyperchloremic metabolic acidosis associated with renal transplantation. *Amer J Med.* 1981;70:786-796.

130. Schwarz C, Benesch T, Kodras K, et al. Complete renal tubular acidosis late after kidney transplantation. *Nephrol Dial Transplant.* 2006;21:2615-2620.
131. Keven K, Ozturk R, Sengul S, et al. Renal tubular acidosis after kidney transplantation–incidence, risk factors and clinical implications. *Nephrol Dial Transpl.* 2007;22:906-910.
132. Jordan M, Cohen EP, Roza A, et al. An immunocytochemical study of H^+ - ATPase in kidney transplant rejection. *J Lab Clin Med.* 1996;127:310-314.
133. Probst P, Soleiman, Zbornay V, Benesch T, Martin Haas M. The effect of kidney transplantation on distal tubular vacuolar H^+-ATPase. *Transplantation.* 2008;85:391-397.
134. Heering P, Grabensee B. Influence of ciclosporin A on renal tubular function after kidney transplantation. *Nephron.* 1991;59:66-70.
135. Heering P, Ivens K, Aker S, Grabensee B. Distal tubular acidosis induced by FK506. *Clin Transplant.* 1998;12:465-471.
136. Tanrisev M, Gungor O, Kocyigit I, et al. Renal tubular acidosis in renal transplant patients: the effect of immunosuppressive drugs. *Ann Transplant.* 2015;20:85-91.
137. Fischer G, Aumuller T. Regulation of peptide bond *cis-trans* isomerisationby enzyme catalysis and its implication in physiological processes. *Rev Physiol Biochem Pharmacol.* 2003;148:105-150.
138. Al-Awqati Q. Terminal differentiation of intercalated cells: the role of hensin. *Annu Rev Physiol.* 2003;65:567-583.
139. Hikita C, Takito J, Vijayakumar S, Al-Awqati Q. Only multimeric hensin located in the extracellular matrix can induce apical endocytosis and reverse the polarity of intercalated cells. *J Biol Chem.* 1999;274:17671-17676.
140. Thies MJW, Mayer J, Augustine JG, Frederick CA, Lilie H, Buchner J. Folding and association of the antibody domain CH3: prolyl isomerisation proceeds dimerization. *J Mol Biol.* 1999;293:67-79.
141. Watanabe S, Tsuruoka S, Vijayakumar S, et al. Cyclosporine A produces distal renal tubular acidosis by blocking peptidyl prolyl cis-trans isomerase activity of cyclophilin. *Am J Physiol Renal Physiol.* 2005;288:40-47.
142. Stransky L, Cotter K, Forgac M. The function of V-ATPases in Cancer. *Physiol Rev.* 2016;96(3):1071-1091.
143. Chen Z, Dong H, Hia Ch, et al. Activation of mTORC1 in collecting ducts causes hyperkalemia. *J Am Soc Nephrol.* 2014;25(3):534-545.
144. Vallon V, Lang F. New insights into the role of serum- and glucocorticoid-inducible kinase SGK1 in the regulation of renal function and blood pressure. *Curr Opin Nephrol Hypertens.* 2005;14:59-66.
145. Gleason CE, Frindt G, Cheng CJ, et al. mTORC2 regulates renal tubule sodium uptake by promoting ENaC activity. *J Clin Invest.* 2014;125(1):117-128.
146. Grahammer F, Nesterov V, Ahmed A, et al. mTORC2 critically regulates renal tubule sodium uptake by promoting ENaC activity. *J Clin Invest.* 2014;125(1):117-128.
147. Morales JM, Wrammer L, Kreis H, et al, Sirolimus European Renal Transplant Study Group. Sirolimus does not exhibit nephrotoxicity compared to cyclosporine in renal transplant recipients. *Am J Transplant.* 2002;2:436-442.
148. McCurdy DK, Frederic M, Elkinton JR. Renal tubular acidosis due to amphotericin B. *N Engl J Med.* 1968;276:124-130.
149. Steinmetz PR, Lawson WJ. Defect in urinary acidification induced in vitro by amphotericin B. *J Clin Invest.* 1970;49:596-601.
150. Sawaya BP, Briggs JP, Schnermann J. Amphotericin B nephrotoxicity: the adverse consequences of altered membrane properties. *J Am Soc Nephrol.* 1995;6:154-164.
151. Rosen S. Turtle bladder. II. Observations on epithelial cytotoxic effect of amphotericin B. *Exp Mol Pathol.* 1970;12:297-305.
152. Gerkens JF, Branch RA. The influence of sodium status and furosemide on canine acute amphotericin B nephrotoxicity. *J Pharmacol Exp Ther.* 1980;214:306-311.
153. Batlle D, Sabatini S, Kurtzman NA. On the mechanism of toluene-induced renal tubular acidosis. *Nephron.* 1988;49:210-218.
154. Streicher HZ, Gabow PA, Moss AH, Kono D, Kaehny WD. Syndromes of toluene sniffing in adults. *Ann Intern Med.* 1981;94(6):758-762.
155. Taher SM, Anderson RJ, Mcartney R, Popovitzer MM, Schrier RW. Renal tubular acidosis associated with toluene "sniffing". *N Engl J Med.* 1974;290:765-768.
156. Camara-Lemarroy CR, Rodriguez-Guitierrez R, Monreal-Robles R, Gonzalez-Gonzalez JG. Acute toluene intoxication- clinical presentation, management and prognosis: a prospective observational study. *BMC Emerg Med.* 2015;15:19.
157. Carlisle EJ, Donnelly SM, Vasuvattakul S, Kamel KS, Tobe S, Halperin ML. Glue-sniffing and distal renal tubular acidosis: sticking to the facts. *J Am Soc Nephrol.* 1991;1(8):1019-1027.
158. Sebastian A, McSherry E, Morris RC Jr. Impaired renal conservation of sodium and chloride during sustained correction of systemic acidosis in patients with type 1, classic renal tubular acidosis. *J Clin Investig.* 1976;58(2):454-469.
159. Sebastian A, McSherry E, Morris RC Jr. Renal potassium wasting in renal tubular acidosis (RTA): its occurrence in types 1 and 2 RTA despite sustained correction of systemic acidosis. *J Clin. Invest.* 1971;50:667-678.
160. Gill JR Jr, Bell NH, Bartter FC. Impaired conservation of sodium and potassium in renal tubular acidosis and its correction by buffer anions. *Clin Sci.* 1967;33:577-592.
161. Dafnis E, Spohn M, Louis B, et al. Vanadate causes hypokalemic distal RTA. *Am J Physiol.* 1992;262(3 Pt 2):F449-F453.
162. Wingo CS, Straub SC. Active proton secretion and potassium absorption in the rabbit outer medullary collecting duct. *J Clin Invest.* 1989;84:361-365.
163. Codina J, DuBose TD Jr. Molecular regulation and physiology of the H^+ K^+ ATPases in kidney. *Semin Nephrol.* 2006;26:345-351.
164. Simpson AM, Schwartz GJ. Distal renal tubular acidosis with severe hypokalemia probably caused by colonic H^+/K^+ ATPase deficiency. *Arch Dis Child.* 2001;84:504-507.
165. Collier DM, Snyder PM. Extracellular protons regulate human ENaC by modulating Na^+ self-inhibition. *J Biol Chem.* 2009;284:792-798.
166. Tanemoto M, Kittaka N, Inanobe A, Kurachi Y. In vivo formation of a proton-sensitive K^+ channel by heteromeric subunit assembly of Kir5.1 with Kir4.1. *J Physiol.* 2000;525(Pt 3):587-592.
167. Pessia M, Imbrici P, D'Adamo MC, Salvatore L, Tucker SJ. Differential pH sensitivity of Kir4.1 and Kir4.2 potassium channels and their modulation by heteropolymerisation with Kir5.1. *J Physiol.* 2001;532:359-367.
168. Guetin V, Vallet M, Jayat M, et al. Renal β-intercalated cells maintain body fluid and electrolyte balance. *J Clin Invest.* 2013;123(10):4219-4231.
169. Finberg KE. The B1-subunit of the H(+) ATPase is required for maximal urinary acidification. *Proc Natl Acad Sci U S A.* 2005;102(38):13616-13621.
170. Lehrmann H, Thomas J, Kim SJ, Jacobi C, Leipziger J. Luminal P2Y2 receptor-mediated inhibition of Na^+ absorption in isolated perfused mouse CCD. *J Am Soc Nephrol.* 2002;13(1):10-18.
171. Satlin LM, Carattino MD, Liu W, Kleyman TR. Regulation of cation transport in the distal nephron by mechanical forces. *Am J Physiol Renal Physiol.* 2006;291(5):F923-F931.
172. Valles P, Farias JM, Batlle D. Inherited proximal and distal renal tubular acidosis. In: *Scientific American Nephrology, Dialysis and Transplantation.* Toronto: Decker; 2017.
173. Batlle D, Hizon M, Cohen E, et al. The use of the urinary anion gap in the diagnosis of hyperchloremic metabolic acidosis. *N Engl J Med.* 1988;318:594-599.
174. Batlle D, Ba Aqeel SH, Marquez A. The urine anion gap in context. *Clin J Am Soc Nephrol.* 2018;13:195-197.

175. Walsh SB, Shirley DG, Wrong OM, Unwin RJ. Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. *Kidney Int.* 2007;71:1310-1316.
176. Walsh S, Turner CM, Toye A, et al. Immunohistochemical comparison of a case of inherited distal renal tubular acidosis (with a unique AE1 mutation) with an acquired case secondary to autoimmune disease. *Nephrol Dial Transplant.* 2007;22:807-812.
177. Han JS, Kim GH, Kim J, et al. Secretory-defect distal renal tubular acidosis is associated with transporter defect in H^+- ATPase and anion exchanger-1. *J Am Soc Nephrol.* 2002;13:1425-1432.
178. Bastani B, Underhill D, Chu N, et al. Preservation of intercalated cell H^+- ATPase in two patients with lupus nephritis and hyperkalemic distal renal tubular acidosis. *J Am Soc Nephrol.* 1997;8:1109-1117.
179. Shayakul C, Jarolim P, Zachlederova M, et al. Characterization of a highly polymorphic marker adjacent to the SCL4A1 gene and of kidney immunostaining in a family with distal renal tubular acidosis. *Nephrol Dial Transplant.* 2004;19:371-379.
180. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U S A.* 2004;101:13368-13373.
181. Santos F, Gil Peña H, Alvarez-Alvarez S. Renal tubular acidosis. *Curr Opin Pediatr.* 2017;29:206-220.
182. Domrongkitchaiporn S, Khositseth S, Stitchantrakul W, et al. Dosage of potassium citrate in the correction of urinary abnormalities in pediatric distal renal tubular acidosis patients. *Am J Kidney Dis.* 2002;39:383-391.
183. Moorthi K, Batlle D. Renal tubular acidosis. In: Gennari J, Adrogue HJ, Galla NE, Madias JH, eds. *Acid-base disorders and their treatment.* Boca Raton (FL): Taylor and Francis; 2005:417-459.
184. Oguejiofor P, Chow R, Yim K, Jaar BG. Successful management of refractory type 1 renal tubular acidosis with amiloride. *Case Rep Nephrol.* 2017;2017:8596169.
185. Sahni V, Rosa R, Batlle D. Potential benefits of alkali therapy to prevent GFR loss: time for a palatable "solution" for the management of CKD. *Kidney Int.* 2010;78(11):1065-1067.
186. Bushinsky DA, Hostetter T, Klaerner G, et al. Randomized, Controlled trial of TRC101 to increase serum bicarbonate in patients with CKD. *Clin J Am Soc Nephrol.* 2018;13(1):26-35.
187. Schmitt MG Jr, Soergel KH, Wood CM, Steff JJ. Absorption of short-chain fatty acids from the human ileum. *Am J Dig Dis.* 1977;22:340-347.
188. Ruppin H, Bar-Meir S, Soergel KH, Wood CM, Schmitt MG Jr. Absorption of short-chain fatty acids by the colon. *Gastroenterology.* 1980;78:1500-1507.