Renal tubular acidosis

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Purpose of review
To facilitate the understanding and knowledge of renal tubular acidosis by providing a summarized information on the known clinical and biochemical characteristics of this group of diseases, by updating the genetic and molecular bases of the primary forms renal tubular acidosis and by examining some issues regarding the diagnosis of distal renal tubular acidosis (RTA) in the daily clinical practice.

Recent findings
The manuscript presents recent findings on the potential of next-generation sequencing to disclose new pathogenic variants in patients with a clinical diagnosis of primary RTA and negative Sanger sequencing of known genes. The current review emphasizes the importance of measuring urinary ammonium for a correct clinical approach to the patients with metabolic acidosis and discusses the diagnosis of incomplete distal RTA.

Summary
We briefly update the current information on RTA, put forward the need of additional studies in children to validate urinary indexes used in the diagnosis of RTA and offer a perspective on diagnostic genetic tests.

Keywords
ammonium, children, metabolic acidosis, renal tubular acidosis

INTRODUCTION

Metabolic acidosis results from the net loss of HCO_3^- or from the net gain of acid. It is diagnosed by a low plasma bicarbonate concentration (<22–24 mEq/l in children and <20–22 mEq/l in infants) and low blood pCO_2 (<40 mmHg in children and <35 mmHg in infants). Metabolic acidosis is usually classified according to the values of serum or plasma anion gap estimated by the difference between the sum of the sodium and potassium ion concentrations minus the sum of the chloride and bicarbonate anion concentrations. Several disorders can give rise to metabolic acidosis in children, usually transient.

GENERAL DESCRIPTION

The term renal tubular acidosis (RTA) refers to a group of diseases characterized by normal serum anion gap or hyperchloremic metabolic acidosis caused by the inability of the renal tubule to retain bicarbonate (HCO_3^-) or to secrete hydrogen ions (H^+) in the presence of normal or moderately impaired glomerular filtration rate [1].

There are four types of primary RTA whose main diagnostic features are summarized in Table 1. Recent comprehensive reviews on these forms of RTA are available in the literature [2▪].

It is of note that the majority of pediatric cases with hyperchloremic metabolic acidosis seen in clinical practice are transient and result from gastrointestinal disorders, such as acute diarrhea.

It should also be kept in mind that, in adults, many forms of RTA are secondary to systemic diseases, such as Sjögren syndrome, systemic lupus erythematosus, rheumatoid arthritis, hypergammaglobulinemia, kidney transplantation and sickle cell disease, or exposure to drugs, such as amphotericin B, lithium carbonate and intravenous bisphosphonates [3] or toxins, whereas most pediatric cases correspond to primary disorders resulting from specific genetic defects in a protein involved in the processes of HCO_3^- reabsorption, HCO_3^- regeneration and H^+ secretion. Primary RTA usually presents early in infancy or early childhood with hyperchloremic metabolic acidosis, persistent if it is not treated, associated with clinical episodes of

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KEY POINTS

- Clinical and biochemical manifestations as well as genetic analysis serve to characterize the four types of primary renal tubular acidosis in children.
- The measurement of urinary ammonium is important for an accurate diagnosis of RTA. Indexes in urine developed to indirectly estimate ammonium need to be validated in infants and children.
- Incomplete forms of distal type 1 RTA can be detected by subtle defects in the ability to acidify the urine. The clinical interest of this diagnosis in pediatric population is questionable.

vomiting, diarrhea, dehydration, polyuria and failure to thrive. Nephrocalcinosis develops early in infants with distal type 1 RTA so that it can be detected by ultrasound within the first weeks of life. In type 4 RTA, the hyperkalemia, often associated with chronic renal failure, may be the most prominent clinical manifestation.

Treatment of RTA patients requires sustained alkali supplementation, habitually provided as sodium bicarbonate or potassium citrate, aiming to normalize blood pH and bicarbonatemia. Adult patients with distal RTA are well controlled by the administration of 2–3 mEq/day of alkali. However, pediatric patients may require higher amounts of bicarbonate due to the alkali needs of growing bones and the transient associated reduction in proximal tubular reabsorption of bicarbonate that may occur in infants with distal RTA. Infants and children with proximal type 2 RTA need much greater amounts of alkali because of their high fractional excretion of bicarbonate. These patients with type 2 RTA usually have generalized proximal tubular dysfunction (i.e. Fanconi syndrome) and therefore require a more complex treatment.

The current review updates recent and updated information on the underlying molecular defect and discusses critical issues for the appropriate diagnosis of primary RTA-based on clinical and biochemical findings.

GENETIC BASIS

Table 2 lists the genes and proteins having loss-of-function mutations known to be involved in the genesis of primary RTA [4▪,5▪▪]. As for distal type 1 RTA, in 10% of 52 cases analyzed in the RenalTube database (www.renaltube.com), a website-based collaborative multicenter effort aimed at the study of primary tubular disorders [6], the underlying pathogenic variant has not been found so far. This requires the confirmation of the clinical diagnosis, sometimes made not on the basis of strict criteria as commented below and also supports the suggestion that additional genes are responsible for the disease.

Table 1. Differential clinical and biochemical characteristics in the four types of primary renal tubular acidosis, which all are hyperchloremic normal serum anion gap persistent metabolic acidosis

		Biochemical features				
RTA	**Clinical manifestations**	**Plasma potassium**	**Urinary acidification[a]**	**Urinary ammonium**	**FE of HCO_3^-**	**Other data**
Type 1, distal RTA	Dehydration. Growth retardation Nephrocalcinosis and urolithiasis [b]Deafness [b]Hemolytic anemia	Low/normal	Defective	Low	Normal	Hypocitraturia, hypercalciuria
Type 2, proximal RTA	In the context of syndromes with generalized proximal tubular dysfunction. Isolated, very rare [b]Ocular anomalies [b]Neurological symptoms	Low/normal	Preserved	Normal	Very high	
Type 3	[b]Osteopetrosis [b]Cerebral calcification	Low/normal	Defective	Low	High	
Type 4	Pseudohypoaldosteronism or hypoaldosteronism	High	Preserved	Low	High	Low GFR

FE of HCO_3^-: urinary fractional excretion of bicarbonate anion with simultaneous normal bicarbonatemia. GFR, glomerular filtration rate.
[a]Assessed by minimal urinary pH achieved in the setting of spontaneous or acid load (NH_4Cl) induced metabolic acidosis or following the administration of furosemide or by urine-blood pCO_2 measured in the presence of normal bicarbonatemia.
[b]Associated in some cases with the RTA, according to the mutated gene responsible for the disease (Table 2).

Table 2. Genetic and molecular basis of the different types of primary renal tubular acidosis

RTA	Mutated gene	OMIM number	Defective protein	Inheritance
Type 1	*ATP6V1B1*	*192132	H^+-ATPase, V1 subunit B1 isoform	AR
	ATP6V0A4	*605239	H^+-ATPase subunit, V0 subunit A4 isoform	AR
	SLC4A1	+109270	Kidney Cl^-/HCO_3^- exchanger (kAE1)	AD or AR
Type 2	*SLC4A4*	*603345	Kidney Na^+/HCO_3^- cotransporter (NBCe1)	AR
	CLCN7	*602727	H^+/Cl^- exchange transporter 7 (CLCN7)	AD with VE
	CA2	*611492	Carbonic anhydrase II	AR
	SLC2A2	*138160	Solute carrier family 2, member 2; (SLC2A2)	AR or AD
	CTNS	*606272	Cystinosin (CTNS)	AR
	FAH	*613871	Fumarylacetoacetate hydrolase (FAH)	AR
	TAT	*613018	Tyrosine aminotransferase (TAT)	AR
	HPD	*609695	4-hydroxyphenylpyruvate dioxygenase (HPD)	AD or AR
	ATP7B	*606882	ATPase, Cu (2+)-transporting, beta polypeptide (ATP7B)	AR
	GALT	*606999	Galactose-1-phosphate uridylyltransferase (GALT)	AR
	CLCN5	*300008	Chloride channel 5 (CLCN5)	XLR
	OCRL	*300535	OCRL gene (*OCRL*)	XLR
Type 3	*CA2*	*611492	Carbonic anhydrase II	AR
Type 4	*NR3C2*	*600983	Nuclear receptor subfamily 3, group C, member 2 (NR3C2)	AD
	SCNN1A	*600228	α subunit of sodium channel, nonvoltage-gated 1 (SCNN1A)	AR
	SCNN1B	*600760	β subunit of sodium channel, nonvoltage-gated 1 (SCNN1B)	AR
	SCNN1G	*600761	γ subunit of sodium channel, nonvoltage-gated 1 (SCNN1G)	AR
	WNK 4	*601844	With-no-lysine kinase member 4 (WNK4)	AD
	WNK1	*605232	With-no-lysine kinase member 1 (WNK1)	AD
	KLHL3	*605775	Kelch-like protein 3 (KLHL3)	AR
	KLHL3	*605775	Kelch-like protein 3 (KLHL3)	AD
	CUL3	*603136	Cullin 3 (CUL3)	AD

Underlined entries corresponds to syndromic forms of proximal type 2 RTA. AD, autosomal dominant; AR, autosomal recessive; OMIM, Online Mendelian Inheritance in Man database; VE, variable expressivity; XLR, X-linked recessive.

Various potential candidate genes for human distal type 1 RTA have been proposed because their inactivation has caused RTA in mice, but there is no evidence that these genes cause the disease in humans [2▪]. Analysis by next-generation sequencing (NGS) of DNA from 10 patients with primary distal RTA disclosed the mutation in nine and suggested that compound heterozygosity of known mutations may be present in exceptional cases [4▪], a hypothesis that needs to be validated by further functional experiments. The extended use of exome sequencing will likely result in the discovery of new genes responsible for RTA, particularly when RTA occurs in association with other apparently unrelated renal or extrarenal manifestations. In this regard, Piret *et al.* [5▪▪] have recently reported a case of proximal RTA secondary to a missense mutation (c.643G > A; p.Gly215Arg) in the gene encoding the chloride/proton antiporter 7 (gene *CLCN7*, protein CLC-7) in a family with autosomal-dominant osteopetrosis associated with renal stones, epilepsy and blindness. As shown in consanguineous families with congenital anomalies of the kidneys and urinary tract, exome sequencing can lead to the detection of syndromes not formerly identified because of atypical phenotypical manifestations or even to the diagnosis of unsuspected primary tubular disorders [7▪▪].

CORRECT CLINICAL DIAGNOSIS OF PRIMARY DISTAL RENAL TUBULAR ACIDOSIS

Type 1 distal RTA is the most common form of primary RTA in Western countries. It is characterized by the inability to maximally decrease urine pH and enhance urinary ammonium (NH_4^+) excretion in the presence of sustained hyperchloremic metabolic acidosis, hypokalemia, early development of nephrocalcinosis and frequent association with nerve deafness [1]. It is of note that primary distal RTA is a permanent condition that cannot be

reliably diagnosed on the basis of an episode of transient metabolic acidosis and simultaneous urinary pH above 5.5, as often occurs [8]. The diagnosis of distal RTA requires the accurate determination of blood pH and bicarbonate concentration by using a blood gas analyzer, the measurement of pH in fresh urine samples using a pH meter and the assessment of titratable acid excretion and, especially, NH_4^+ in urine collected during acidosis. The amount of titratable acid is more or less fixed but the normal kidneys respond to chronic metabolic acidosis by markedly increasing the elimination of NH_4^+. In distal RTA, the NH_4^+ excretion is reduced due to impaired trapping of ammonia (NH_3) in the collecting duct subsequent to the defective H^+ secretion [3]. Unfortunately, the determination of urinary NH_4^+ is not available in the majority of hospital clinical laboratories, and, subsequently, appropriate reference normative data for the elimination of NH_4^+ in acidotic children with healthy kidneys are not available. Indices proposed to indirectly estimate the amount of NH_4^+ in urine, such as the urine anion gap or osmolal gap, provide a rough estimation of the amount of NH_4^+, have limitations in the clinical interpretation and, in addition, are not frequently reported in publications describing pediatric cases of RTA. Urine anion gap is calculated by the formula $Na^+ + K^+ - Cl^-$, and negative values are supposed to reflect high concentrations of urinary NH_4^+ unless significant amounts of bicarbonate, for instance, if the urinary pH is greater than 6.5, or other organic anions are present in the urine [9]. Sulyok and Guignard [10] showed a poor correlation between urinary NH_4^+ and urinary anion gap in neonates and infants during the first weeks of life. Additional studies are necessary in pediatric population to analyze this correlation in different clinical situations. Likewise, a urinary osmolal gap, calculated by the formula:

$$\text{Measured urine osmolality} - 2(Na^+ + K^+) + \frac{\text{urea nitrogen (mg/dl)}}{2.8} + \frac{\text{glucose (mg/dl)}}{18}$$

with a value below 100 has been proposed as indicative of defective NH_4^+ elimination in metabolic acidosis [11] but more clinical studies are needed to confirm whether the urinary osmolality gap is an appropriate index of urinary NH_4^+ concentration in infants and children. Unpublished preliminary data from our hospital's clinical laboratory indicate that technical modifications proposed to facilitate the measurement of NH_4^+ in urine [12] can reliably be used in samples from pediatric patients.

INCOMPLETE DISTAL RENAL TUBULAR ACIDOSIS

Normal kidneys respond to metabolic acidosis, either spontaneous or induced by ammonium chloride or another acidifying agent, by decreasing urinary pH below 5.5. The ability to acidify the urine can also be explored by administration of furosemide that does not cause acidosis but augments distal nephron sodium delivery and increases the lumen-negative electrical gradient favoring H^+ secretion [1]. The inability of the kidneys to achieve a urine pH lower than 5.5 is usually considered as a defect in urinary acidification and indicative of underlying incomplete distal RTA in patients without spontaneous metabolic acidosis or acidemia. This assumption has mostly been made in adults with osteoporosis, chronic interstitial nephritis, kidney calcium phosphate stones, medullary sponge kidney or lithium therapy [13,14]. Shavit *et al.* [15▪] have recently proposed that a normal response to the furosemide + fludrocortisone test [16] can be used as a screening method to exclude distal RTA in adult patients with recurrent kidney stone formation or nephrocalcinosis, whereas an abnormal urinary acidification following furosemide + fludrocortisone administration needs to be confirmed by a subsequent ammonium chloride test. In children, Sharma *et al.* [17] diagnosed incomplete distal RTA by the coexistence of blood pH greater than 7.30, serum bicarbonate greater than 18 mEq/l and urinary pH higher than 5.5 despite systemic acidosis induced by oral ammonium chloride in 17 out of 40 Indian patients who underwent surgical correction of posterior urethral valves. The height of these children improved with sustained sodium bicarbonate treatment. Thus, there are patients with acquired tubulointerstitial kidney diseases who are unable to maximally acidify the urine when challenged by different acidifying stimulus but do not develop metabolic acidosis. Some individuals do not acidify the urine after furosemide + fludrocortisone but they do it normally when challenged by ammonium chloride [15▪]. This raises the issue of urinary acidification needing to be carefully assessed in the clinical setting, and factors such as urine osmolality as well as sodium and NH_4^+ concentrations need to be taken into consideration for a correct interpretation of the ability to achieve a minimal urinary pH.

It is known that the measurement of urine pCO_2 when the urinary pH is higher than that of blood is a sensitive index of distal nephron H^+ secretion so that the urine minus blood pCO_2 difference should be greater than 20 mmHg in normal individuals [3,18]. Interestingly, Zhang *et al.* [19] recently reported two members of a family heterozygous for a mutation in the H^+-ATPase, V1 subunit B1

who did not achieve a normal pCO_2 gradient when they were infused with intravenous sodium bicarbonate to increase their urine pH above 7.0 and did not decrease the urine pH as much as the wild-type relative after receiving ammonium chloride. They also had hypocitraturia and hypercalciuria. Based on complementary in-vitro functional studies, they proposed the hypothesis that heterozygous carriers of mutations known to cause autosomal recessive primary distal RTA (Table 2) might have incomplete distal RTA. Even if this were true, clinical interest in detecting subtle urinary acidification defects in relatives of individuals with primary forms of distal RTA or in pediatric patients with acquired interstitial renal disorders is rather questionable.

CONCLUSION

RTA diagnosis must be based on clinical and biochemical manifestations. Blood acid–base equilibrium, urine pH and NH_4^+ excretion requires careful interpretation, and indirect estimates of urinary values of NH_4^+ need validation in a pediatric population. Incomplete forms of distal type1 RTA disclosed by inability to maximally urine acidification can be found in patients with chronic interstitial kidney disorders, although the clinical interest of this finding in children remains to be established. Nowadays, the diagnosis of congenital primary forms of RTA requires the identification of the underlying molecular defect. Studies by NGS will likely disclose new genes responsible for the disease.

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Conflicts of interest

There are no conflicts of interest.

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