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The pathophysiology of distal renal tubular acidosis

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Abstract | The kidneys have a central role in the control of acid-base homeostasis owing to bicarbonate reabsorption and production of ammonia and ammonium in the proximal tubule and active acid secretion along the collecting duct. Impaired acid excretion by the collecting duct system causes distal renal tubular acidosis (dRTA), which is characterized by the failure to acidify urine below pH 5.5. This defect originates from reduced function of acid-secretory type A intercalated cells. Inherited forms of dRTA are caused by variants in *SLC4A1, ATP6V1B1, ATP6V0A4, FOXI1, WDR72* and likely in other genes that are yet to be discovered. Inheritance of dRTA follows autosomal dominant and recessive patterns. Acquired forms of dRTA are caused by various types of autoimmune diseases or adverse effects of some drugs. Incomplete dRTA is frequently found in patients with and without kidney stone disease. These patients fail to appropriately acidify their urine when challenged, suggesting that incomplete dRTA may represent an intermediate state in the spectrum of the ability to excrete acids. Unrecognized or insufficiently treated dRTA can cause rickets and failure to thrive in children, osteomalacia in adults, nephrolithiasis and nephrocalcinosis. Electrolyte disorders are also often present and poorly controlled dRTA can increase the risk of developing chronic kidney disease.

Glossary

Endolymph: Potassium rich fluid filling the cochlear duct and membranous labyrinth of the inner ear, secreted by the stria vascularis.

Ovalocytosis: red blood cell deformity with oval-shaped red blood cells, also called elliptcytosis, that are mostly caused by defects in the cytoskeleton. In the case of SAO, the anchoring of the cytoskeleton to the membrane is reduced due to the absence of the AE1 containing protein complex.

Sjögren overlap syndrome. Overlap of autoimmune disorders with anti-SSA(Ro) positive antibodies that may include features of Sjögren, SLE, myositis, scleroderma, vasculitis and rheumatoid arthritis.

[H1] Introduction

Acid-base homeostasis is critical for the normal functions of cells and organs and is maintained by the lungs (respiration), kidneys (acid excretion) and other organs, including bone, liver and skeletal muscle. The central role of the kidneys in maintaining long-term acid-base homeostasis is evident from rare inherited disorders of renal acidification, known as tubular renal acidosis, and more common forms of renal acidosis that are seen in patients with advanced chronic kidney disease (CKD).

Impairment of renal function can cause metabolic acidosis. Based on the predominant mechanism different subtypes of renal (tubular) acidosis (RTA) can be distinguished. Type I RTA is of distal origin (dRTA) and causes reduced urinary acidification and ammonium excretion. Impaired proximal tubule bicarbonate reabsorption with preserved urinary acidification is the hallmark of type II proximal renal tubular acidosis (pRTA) [1]. Type III RTA comprises impaired proximal and distal tubular functions and can be seen as a mixed type I and II RTA. Whether type III is an independent form of RTA has been debated. Type IV RTA is hyperkalemic in contrast to type I and II RTA and caused by a failure of aldosterone secretion or signaling [2]. The renal acidosis observed in patients with CKD is different from classic renal tubular acidosis and includes hyperkalemia and a failure of the proximal tubule to generate bicarbonate from ammoniagenesis but has preserved ability to acidify urine [3,4].

Distal renal tubular acidosis (dRTA) can occur early in life, likely owing to mutations, or later in life, mostly owing to acquired conditions. Few data are available to estimate the prevalence of dRTA. An analysis of the UK Clinical Practice Research Datalink database estimated a prevalence of 0.46 recorded cases and 1.60 suspected or recorded cases per 10,000 people. Approximately 22% of recorded cases and 7.6% of suspected or recorded cases were considered to be primary dRTA[5]. A US study that used employer-sponsored insurance data estimated a prevalence of 0.38 patients with a diagnosis of primary dRTA and 3.88 patients with a diagnosis of acquired dRTA per 100,000 people[6,7].

Acidosis might promote the progression of CKD[8,9] and patients with primary forms of dRTA may be at increased risk of developing CKD, warranting early diagnosis and monitoring. Whether CKD in patients with dRTA is a consequence of acidosis or of other associated pathologies, such as nephrolithiasis or calcinosis, and whether correction of acidosis alone is sufficient to prevent CKD in these patients remains to be established. Physicians should also be aware of other conditions that are associated with dRTA such as progressive hearing loss and the secondary consequences of acidosis on bone growth and health as well as electrolyte balance.

In the past 25 years, progress has been made in our understanding of the genetics, cellular pathomechanisms and clinical features of dRTA. In this Review, we summarize the role of the kidneys in acid excretion with a focus on intercalated cells. We highlight the roles of the genes involved in primary forms of dRTA and detail the molecular and cellular mechanisms by which pathogenic variants in these genes cause dRTA and renal and extrarenal manifestations. We also discuss acquired and incomplete forms of dRTA.

[H1] Role of the kidneys in acid-base homeostasis

A healthy adult with a mixed balanced diet and no systemic or acute disease produces approximately 1 mEq of acid per kg body weight per day, (i.e. ~70 mEq of acid per day in a 70 kg person)[10]. This acid load derives mostly from the metabolism of animal protein, which produces non-volatile acids that must be excreted via the kidneys. By contrast, volatile acids, mostly CO_2, that are produced by metabolism of carbohydrates, proteins or lipids can be exhaled.

Kidneys contribute to the control of acid-base homeostasis by reabsorbing filtered bicarbonate (~4500–5000 mEq per day), regenerating bicarbonate through ammoniagenesis (~40 mEq per day) and excreting acids in the form of free protons, ammonium and titratable acids (mainly phosphate). Several nephron segments are involved in renal acid-base handling, including the proximal tubule, thick ascending limb of the loop of Henle, connecting tubule and cortical and medullary collecting ducts.[11-15]

The collecting system of the nephron consists of the late distal convoluted tubule (DCT2), connecting tubule, cortical collecting duct, outer medullary collecting duct (OMCD) and inner medullary collecting duct (IMCD). These segments are composed of several distinct cell types. Segment-specific cells (also known as principal cells) are mostly involved in reabsorbing Na^+ and water and excreting K^+. These cells are characterized by expression of the epithelial Na^+ channel (ENaC), the ATP-sensitive inward rectifier potassium channel 1 (ROMK, also known as KCNJ1) and the aquaporin 2 (AQP2) and aquaporin 3 (AQP3) water channels[16].

The second major cell type in the collecting system is intercalated cells, which can be subdivided into at least two main subtypes: type A acid-secretory intercalated cells (also known as α-intercalated cells) and type B bicarbonate-secreting intercalated cells (also known as β-intercalated cells) (**Figure 1**). In type A intercalated cells, cytosolic carbonic anhydrase II (CAII) facilitates the conversion of CO_2 and H_2O into H^+ and HCO_3^-. H^+ is secreted into urine via apically expressed H^+-ATPases[17] and HCO_3^- is released into the blood by the basolaterally located anion exchange protein 1 (AE1, also known as SLC4A1). In the kidney, AE1 is exclusively expressed in type A intercalated cells[18]. Acid excretion by type A intercalated cells accounts for approximately 30 mEq of acid per day, thus completing the removal and buffering of acids from normal metabolism.

Type B intercalated cells also generate HCO_3^- and H^+ via CAII. In these cells, HCO_3^- is secreted into the urine by the luminally located Cl^-/HCO_3^- exchanger, pendrin (also known as SLC26A4), whereas H^+ is pumped into the blood by basolateral H^+-ATPases (also known as V-ATPases). Pendrin is a specific marker of type B intercalated cells in the kidney[19]. A third subtype of intercalated cells, non-A/non-B intercalated cells, has also been identified [20]. These cells express pendrin and H^+-ATPases at their

luminal side, resulting in net chloride reabsorption. Whether they represent a particular state of type B intercalated cells or a distinct cell type remains unclear. Nevertheless, pendrin-expressing cells not only participate in acid-base homeostasis but also have an important role in controlling salt balance and blood pressure[21-24].

The developmental origin of intercalated cells has not been fully elucidated. This origin is of interest because the collecting duct system has a high degree of plasticity and adapts to changes in systemic electrolyte and acid-base status. During chronic acidosis or acid-loading, the relative number of type A intercalated cells increases, possibly at the expense of type B intercalated cells.[25,26] By contrast, during chronic alkalosis or alkali-loading, the relative number of type B intercalated cells increases and the number of type A intercalated cells is reduced[27]. During development or normal replacement of collecting duct cells, AQP2-expressing cells might serve as precursors of all subtypes of intercalated cells[28]. Differentiation of AQP2-expressing precursors towards the intercalated cell lineage might be regulated by the forkhead box protein I1 (FOXI1) transcription factor and NOTCH signaling, involving JAG, NOTCH1 and NOTCH2 (**Figure 2**).

In mice, absence of Notch or Foxi1 signaling leads to the predominant appearance of cells that concurrently express markers of both principal and intercalated cell lineages and the development of dRTA[29]. Transcription factor CP2-like protein 1 (TFCP2L1) mediates some of the downstream effects of FOXI1, repressing transcripts that are typical of principal cells and inducing genes that are specific to intercalated cells[30]. Similarly, absence of Adam10 shifted the differentiation of AQP2-expressing precursors from principal cells towards intercalated cells in mice[31]. Whether any of these factors also have a role in remodeling of the collecting duct in response to acid or alkali has not been determined. Various signalling molecules have been implicated in this adaptive remodeling, including growth/differentiation factor 15 (GDF15)[32], hensin (DMBT1)[33], galectin-3[34] and β1-integrin[35] as well as hypoxia-inducible factor 1-alpha (HIF1α)–stromal cell-derived factor 1 (SDF1, also known as CXCL12)–C-X-C chemokine receptor type 4 (CXCR4) signaling[36]. Loss of these signalling pathways causes dRTA in various animal models; hence, the encoding genes might be considered candidate genes for ‘orphan’ cases of dRTA.

[H1] Primary forms of dRTA

Primary (also known as inborn) forms of dRTA are caused by mutations in genes that are required for normal acid excretion by the collecting duct (**Table 1**). Some of these genes are also expressed outside of the collecting duct, resulting in extrarenal manifestations in some forms of primary dRTA.

[H2] SLC4A1

Variants in *SLC4A1*, which encodes AE1, can cause autosomal dominant and autosomal recessive forms of dRTA that are not associated with sensorineural deafness[37-39]. AE1 is expressed in a long form in red blood cells and in a short form, kAE1, in the kidney. kAE1 lacks the first 65 amino acids (NH_2-terminal) of full-length AE1[40]. Mutations in *SLC4A1* can cause hereditary forms of hemolytic anemia and/or dRTA with some variants causing both diseases. dRTA due to dominant *SLC4A1* variants is usually diagnosed late in infancy or in adulthood, whereas recessive *SLC4A1* disease is typically diagnosed earlier in life[41,42]. The most frequent recessive variant, G701D, causes dRTA and red blood cell defects. A series of *SLC4A1* variants that have been mostly identified in patients from South-East Asia are associated with dRTA and ovalocytosis **[G]**.[43] These variants are known as South Asian ovalocytosis (SAO) mutations. In the white population, R589H is the most common *SLC4A1* variant that leads to dominant dRTA[44].

The impact of *SLC4A1* mutations on the functions of kAE1 has been examined in polarized and non-polarized cell systems and transgenic mouse models. Mice that lack *Slc4a1* present with severe dRTA that may be aggravated by excessive hemolysis and the anemia that is present in these animals[45]. The mice also have nephrocalcinosis on a background of alkaline urine, hypocitraturia, hypercalcuria and hyperphosphaturia[46]. The intercalated cells of cortical collecting ducts isolated from mice that lacked Slc4a1 showed a 50% reduction in basolateral chloride/bicarbonate exchanger activity, suggesting the presence of other anion exchangers that partially compensate for loss of Ae1 function(s). One such anion exchanger might be *Slc26a7*.

The R607H knock-in mouse model mimics the human R589H *SLC4A1* variant[47]. Heterozygous and homozygous R607H mice had incomplete dRTA with a reduced

number of type A intercalated cells but preserved targeting of mutant Ae1 to the basolateral membrane. Consistent with findings in kidney biopsy samples from patients with other dominant *SLC4A1* variants, targeting of H^+-ATPase to the luminal membrane of type A intercalated cells seemed to be reduced. In addition, intercalated cells accumulated ubiquitinated material, suggesting impairment of the degradative pathway in the absence of normal AE1[47].

Most kAE1 variants cause either retention of mutant protein in the endoplasmic reticulum or Golgi with direct routing to lysosomes via late endosomes or reduced stability and half-life of the mutant protein after being trafficked to the basolateral membrane [48,49] (**Figure 3A**). Some variants, including R589H, also have reduced transport activity assessed in red blood cells[50] and in heterologous cell systems[51]. The R589H variant was initially shown to be mostly retained in the endoplasmic reticulum, with some transporters being mistargeted to the apical membrane in the polarized MDCK model system [48].

Another group of pathogenic variants affect the COOH-terminal end of kAE1. Some researchers have reported that these variants lead to apical insertion of mutant transporters in cell culture models[52,53], whereas others have not found apical mistargeting but reported intracellular retention and accelerated degradation similar to other variants[54,55]. A major limitation of these studies is that current cell models only partly reflect intercalated cell phenotypes and no kidney biopsy samples from patients with these variants have been analysed to date. The COOH-terminal end of kAE1 interacts with Na^+/K^+-ATPases and seems to be important for expression of the pump at the basolateral side[56]. However, the importance of Na^+/K^+-ATPases for transport in intercalated cells is unclear. Immunohistochemistry data suggest that the abundance of this pump is low in intercalated cells compared to other cells along the nephron[57]. Moreover, functional data demonstrate that overall transport activity of intercalated cells is energized by H^+-ATPases[58].

Mistargeting of mutant AE1 causes a defect in polarized cells such as intercalated cells but does not affect membrane insertion in non-polarized red blood cells. Many kAE1 variants are able to interact with the chaperone protein glycophorin A that recruits mutant AE1 to the plasma membrane[59]. Glycophorin is abundantly expressed in red blood cells but absent from intercalated cells[59]. Thus, the absence of a major

red blood cell phenotype in patients who have dRTA owing to kAE1 variants is likely due to a combination of factors, including the presence of glycophorin A, which rescues variants that would otherwise be retained in the endoplasmic reticulum or Golgi, and the fact that red blood cells are non-polarized so are not affected by mistargeting of variants that might be aberrantly inserted into the apical membranes of intercalated cells.

Several kAE1 variants that cause autosomal recessive dRTA, such as S773P and G701D, induce large cation leaks when expressed in oocytes. Such cation leaks could contribute to red blood cell pathologies and at least partly explain the impact of the recessive G701D variant on hemolysis[60].

An analysis of kidney tissue sections from a patient with SLC4A1-dRTA owing to a S613F variant identified very few intercalated cells and those that were present had diffuse kAE1 and H^+-ATPase immunostaining and were hypopmorphic[61] (**Figure 3B**). Likewise, in kidney tissue sections from two patients with the G609R variant, kAE1 was almost absent with very few cells stained in a diffuse pattern and H^+-ATPase staining was mostly cytosolic[62]. Thus, in human kidney, mutations in kAE1 might be associated with a reduced number of intercalated cells with impaired functions. In the cases of the S613F and G609R variants, diffuse intracellular staining may be consistent with a trafficking defect of mutant proteins to the basolateral membrane. Similar findings in the R607H mouse model may suggest a class effect of these mutations[47].

[H2] ATP6V1B1, ATP6V0A4 and ATP6V1C2

Variants in *ATP6V1B1, ATP6V0a4* and *ATP6V1C2*, which encode the B1, A4 and C2 subunits of H^+-ATPases, respectively, have been associated with dRTA. H^+-ATPases are multimeric proteins consisting of a membrane embedded V_0 domain and a cytosolic V_1 domain connected by a stalk. V_1 binds and hydrolyzes ATP while V_0 forms the pore for H^+-transfer. In the human genome, at least 43 genes encode various subunits of the H^+-ATPase, some of which have multiple isoforms[63]. Additional accessory subunits modify H^+-ATPase function[64]. In most cell types, H^+-ATPases are found in intraorganellar membranes (i.e., in lysosomes, endosomes, Golgi apparatus and neurotransmitter-containing vesicles). However, they are expressed at the plasma

membrane in some specialized cell types, including renal intercalated cells, proximal tubule cells, osteoclasts, sustentacular cells of the olfactory mucosa, clear cells in the epididymis, activated macrophages and cells of the stria vascularis in the inner ear. Specific isoforms of the B, A, D, and C subunits are found only in a subset of specialized cells and only in pumps with distinct subcellular localization, explaining how mutations in single H^+-ATPase genes can give rise to organ-specific pathologies as in the case of variants in *ATP6V1B1 and ATP6V0A4*[17,65].

ATP6V1B1-associated and *ATP6V0A4*-associated dRTA are inherited in an autosomal recessive manner and associated with a variable degree and prevalence of sensorineural deafness[66,67]. The combination of symptoms is explained by enriched expression of these genes in the intercalated cells and structures of the inner ear . Variants in *ATP6V1B1* or *ATP6V0A4* account for ~50-60% of primary dRTA in various cohorts[68-71]. These variants can be homozygous or compound heterozygous with mostly missense and nonsense mutations. A higher prevalence of patients with homozygous variants is found in countries or societies with higher rates of consanguineous marriages. Patients with dRTA due to *ATP6V1B1* or *ATP6V0A4* are typically diagnosed during their first year of life, which probably reflects more severe symptoms than those of patients with *SLC4A1*-related dRTA.

In the kidney, *ATP6V1B1* is highly expressed in intercalated cells but some expression is also found in the thick ascending limb of the loop of Henle and in the distal convoluted tubule[72]. Outside the kidney, *ATP6V1B1* is expressed in clear cells of the epididymis, sustentacular cells, some lung cells and in cells lining the endolymphatic sac of the inner ear[65]. The impact of loss of *Atp6v1b1* has been examined in mouse kidney[83]. Both isoforms of the b subunit (b1 and b2) are present in murine intercalated cells. The b1 isoform is enriched in intercalated cells and associated with the plasma membrane, while the ubiquitously expressed b2 isoform is found predominantly in a cytosolic location. In the absence of b1, b2 relocalizes to the apical plasma membrane. This finding may explain why intercalated cells from Atp6v1b1-deficient mice have some residual plasma membrane H^+-ATPase activity. However, b2 is not able to support normal H^+-ATPase function. Mice that lack the b1 subunit do not adapt appropriately to an acid load and cannot increase their H^+-ATPase activity in the intercalated cell plasma membrane. Likewise, intercalated cells that lack the b1 subunit do not respond to angiotensin II, which is a potent stimulus for intercalated cell

H^+-ATPase activity, suggesting that b2 is able to support some basal H^+-ATPase activity but pumps lacking the b1 isoform cannot respond to physiological stimuli[73,74].

Expression of human mutant B1 subunits in a mammalian cell line and in yeast demonstrated that these mutant proteins fail to produce functional proton pumps due to an impairment in trafficking or pump assembly[75,76] (**Figure 3C**). When challenged with an acid load, healthy people show increased excretion of B1 but not B2 H^+-ATPase subunits in urinary extracellular vesicles[77,78]. In patients with dRTA, B1 is barely detectable in urinary vesicles and excretion of B1 and B2 does not increase in response to an acid challenge. This finding is consistent with the results of studies in patients with biopsy-proven absence of the A subunit of H^+-ATPases and of more detailed studies in animal models [77,78].

The A4 H^+-ATPase subunit is expressed in intercalated cells and in proximal tubule cells where it localizes to the brush border membrane and endolysosomal system[79-82]. Atp6v0a4-knockout mice exhibit albuminuria and low molecular weight proteinuria with an altered structure of the endolysosomal apparatus and accumulation of endocytic material[80]. As acidosis can induce changes in proximal tubular metabolism and function, whether proximal tubular dysfunction in patients with ATPV0A4 variants occurs independently of their acid-base status remains to be investigated[83]. In addition to impairments in H^+-ATPase assembly, trafficking or activity[84], mutations in the A4 subunit may reduce interactions of the pump with other proteins. The A4 subunit mediates interactions with the glycolytic enzyme phosphofructokinase 1[85] and glycolysis is an important energy source that supports H^+-ATPase activity in intercalated cells[86].

A homozygous missense variant in ATP6V1C2 was identified in a patient with hypokalemic metabolic acidosis and alkaline urine who died at an early age due to kidney failure[87]. Single cell transcriptome data from mouse kidney showed that this subunit is highly enriched in intercalated cells, supporting a role in dRTA[88]. Functional analysis of the mutated C2 subunit in yeast complementation assays suggested that the mutation impaired H^+-ATPase activity[87]. However, the importance of this finding is unclear because only one patient has been identified to date and kidney failure is a very uncommon finding in dRTA. Moreover, biallelic protein-changing gene variants in ATP6V1C2 that do not cause overt pathogenicity are common, and the *ATP6V1C2*

variant identified in the patient with dRTA is more common in the general population than would be expected for this rare disorder[89]. Thus, strong supporting evidence for a role of *ATP6V1C2* in dRTA is missing. The identification of more patients with dRTA who have causative variants in this gene is required to confirm its role in this disorder.

[H2] FOXI1

Three patients with dRTA from two consanguineous families with two distinct missense variants in FOXI1 have been identified to date[90]. The patients were homozygous for these variants and were diagnosed with hypokalemic hyperchloremic dRTA, bilateral nephrocalcinosis and early-onset sensorineural deafness treated with cochlear implants. Deafness was associated with an enlarged aqueduct. Notably, siblings who were heterozygous for the missense variants had no apparent hearing impairment. This finding is important because heterozygosity for *FOXI1* mutations has been speculated to cause hereditary hearing loss[91]. All three patients also had medullary cysts, which are a common feature in all genetic forms of dRTA[92,93]. Concomitant ablation of Foxi1 abrogated cyst formation in a mouse model of tuberous sclerosis, suggesting that the absence of FOXI1 protects against cyst formation rather than causes kidney cysts[94].

A Chinese patient with congenital deafness and enlarged vestibular aqueduct who was compound heterozygous for two variants in *FOXI* has also been described. The *FOXI* variants were both likely pathogenic and induced an in-frame duplication and a missense variant. However, the variants were not functionally tested and whether the patient had dRTA was not reported[95].

Two missense variants in *FOX1* (p.L146F and p.R213P) that were identified in patients with dRTA and deafness are predicted to affect DNA binding by the transcription factor. In transfected cells, the mutated FOX1 proteins did not bind DNA and failed to activate typical target genes[90]. Thus, both variants are expected to lack the ability to induce differentiation of cells in the collecting duct and activate the transcription of essential genes required for renal acid excretion.

FOXI1 is expressed in all subtypes of intercalated cells[96], in the endolympathic sac of the inner ear, in clear and narrow cells of the epididymis and in cystic fibrosis transmembrane conductance regulator (CFTR)-expressing pulmonary ionocytes[29,97-100]. Although loss of Foxi1 reduced Cftr expression in mouse lung, the role of Foxi1 in

lung in mice and humans remains to be established. Foxi1 target genes in the kidney, inner ear and epididymis include pendrin, Ae1, Ae4, and the a, b1, e2 and a4 H^+-ATPase subunits[29,97]. The expression of these genes is very low in mice that lack Foxi1. Foxi1-deficient mice develop hyperchloremic dRTA and deafness and the males are infertile[97]. Lack of Foxi1 impairs terminal differentiation of the collecting duct epithelium with all cells co-expressing markers of principal and intercalated cells[29].

[H2] WDR72

Variants in *WDR72* have been detected in patients with amelogenesis imperfecta, a defect in tooth mineralization and enamel formation that is inherited in an autosomal recessive manner[101]. These patients also have dRTA and multiple families have been identified over the last few years [102-104]. Patients carry homozygous or compound heterozygous missense or truncating variants that are predicted to impair protein function[102-104]. Hearing deficits have not been reported in patients with WDR72-dRTA.

The molecular and cellular mechanisms of WDR72-dRTA and the function of WD repeat-containing protein 72 (WDR72) are unknown. WDR72 mRNA is highly enriched in all subtypes of intercalated cells in the kidney[88]. Bone and teeth are also major sites of WDR72 expression[101]. WDR72 is a member of the WD40-repeat protein family. Other members of this family are often involved in coordination of multi-protein complexes. WDR72 is related to WD-repeat containing protein 7 (WDR7, also known as rabconnectin-3 β or TRAG), which is involved in the Ca^{2+}-dependent trafficking and exocytosis of synaptic neurotransmitter vesicles[105,106]. WDR7 can bind to H^+-ATPase subunits[107] and other members of this gene family are required for vesicular trafficking and endovesicular acidification in neurons, suggesting that WDR72 might have a role in H^+-ATPase trafficking and/or assembly in intercalated cells (**Figure 3C**).

In genome wide association studies, *WDR72* was associated with kidney stones[108,109], more alkaline urine [109], lower estimated glomerular filtration rate (eGFR)[110], CKD risk[111,112], lower urinary uromodulin levels indexed to creatinine[113] and susceptibility to scrub typhus[114]. Whether these associations are linked to a potential role of WDR72 in urinary acidification remains to be established.

[H2] Orphan dRTA

In about 20-25% of children with a diagnosis of dRTA, causative variants cannot be identified but a genetic basis is likely. Variants in the non-coding regions of established dRTA genes or in the coding or non-coding regions of additional genes may cause dRTA in these patients. Animal studies have identified several candidate genes that cause incomplete or complete dRTA when deleted or mutated in mouse models. These genes include the K^+/Cl^--cotransporter KCC4 (*SLC12A7*)[115], the anion exchanger *SLC26A7*, the ammonia transporters RhGB (*SLC42A2*) and RhCG (*SLC42A3*)[116], hensin (*DMBT1*), *TFCP2L1, galectin-3, CXCL12, CXCR4*, carbonic anhydrase IV and various subunits of the H^+-ATPase enriched in intercalated cells. Advances in exome-sequencing and whole genome sequencing are likely to lead to the identification of additional genes that cause dRTA and of causative variants in children with dRTA who currently lack a genetic diagnosis.

[H1] Acquired forms of dRTA

Acquired dRTA can be caused by nephrocalcinosis of any cause and by various drugs or toxins. Nephrocalcinosis and dRTA often coexist and dRTA can cause nephrocalcinosis and vice versa. Nephrocalcinosis is mostly if not exclusively medullary and causes impaired urinary acidification by mechanisms that might involve direct damage to the collecting duct and/or local inflammation.

However, by far the most common cause is autoimmune disease, most frequently Sjögren or Sjögren overlap syndrome **[G]** (**Table 2**). Renal tubular acidosis is also a common finding in patients with sickle cell disease.

[H2] Sjögren syndrome

Sjögren syndrome is characterized by inflammation of lacrimal and salivary glands causing sicca syndrome and patients are positive for anti-SSA (Ro) and anti-SSB (La) antibodies[117]. Renal involvement is variable and can include tubulointerstitial nephritis, electrolyte disorders (mostly hypokalemia and hyperchloremia), glomerular disease, Fanconi syndrome or dRTA. Kidney disease is present in about one-third of patients with primary Sjögren syndrome[117].

The prevalence of dRTA in Sjögren syndrome is estimated to be around 5-25%[188-190]. However, a study of 130 patients with primary Sjögren's syndrome and renal

involvement who were admitted to a Chinese hospital reported a prevalence of dRTA of 73%[156]. Another Chinese study that used nationwide registry data described 4,479 patients with Sjögren syndrome of whom 257 had dRTA and 4222 had no renal involvement[117-121]. Autoantibodies against kidney structures are a variable finding in patients with Sjögren syndrome and dRTA and may be directed against intercalated cells. CAII and the B1 H^+-ATPase have been suggested to be targets of these autoantibodies[122-124] and immunization of mice with CAII induces Sjögren-like sialoadenitis [125] and dRTA[126]. However, pharmacological inhibition or genetic deletion of CAII causes a mixed type of proximal and distal RTA and direct binding of autoantibodies to H^+-ATPase subunits remains to be demonstrated. T cell infiltrates can often be seen in kidney biopsy samples from affected patients.

[H2] Other autoimmune diseases

Rheumatoid arthritis, primary biliary (sclerosing) cholangitis (PBC), systemic lupus erythematosus (SLE), and tubulointerstitial nephritis have also been associated with dRTA[118,127,128]. One study that included 18 patients with PBC reported a prevalence of dRTA of 33%[127]. In patients with PBC, dRTA is associated with tubulointerstitial nephritis[129]. A kidney biopsy sample from a patient with PBC and dRTA showed an absence of intercalated cells and their serum stained a subset of cells along the collecting duct, suggesting the presence of autoantibodies against these cells[128]. The true prevalence of dRTA in patients with SLE is unknown but appears to be rare. SLE and Sjögren syndrome may also overlap in some patients. dRTA is often recognized only after severe hypokalemia has developed[130].

A subset of patients with tubulointerstitial nephritis have IgM-secreting CD138-positive plasma cell infiltrates in kidney biopsy samples [131]. A study of 13 such patients reported that all had dRTA, 92% had signs of proximal tubule damage (Fanconi syndrome), 82% had anti-mitochondrial antibodies, 46% had PBC and 31% had Sjögrens syndrome. All patients had eGFR <60 ml/min/1.73 m^2 and kidney biopsy samples from some patients showed reduced expression of H^+-ATPase subunits, AE1 and H^+,K^+-ATPases[131]. Whether this form of tubulointerstitial nephritis represents a distinct subtype of TIN or a continuum of related diseases such as Sjögrens syndrome and PBC requires further investigation.

[H2] Sickle cell disease

Renal tubular acidosis is common in patients with Sickle cell disease (SCD). In a cohort of 441 patients, 42% had acidosis with reduced urinary ammonium excretion, normal aldosterone and a urine pH around 5.5 [132]. In another cohort of 25 patients, 52% had an abnormal furosemide and fludrocortisone (F+F) test but only 16% had overt metabolic acidosis[133]. High hemolytic activity and ischaemic renal damage might be risk factors for metabolic acidosis in patients with SCD.

[H2] Drugs and toxins

dRTA can occur as an adverse effect of several commonly prescribed drugs (Table 3) or as a result of exposure to various toxins.

[H3] Lithium. About 50% of patients who receive lithium experience some kidney adverse effects and a subset develop acidosis with alkaline urine. The strongest risk factors for kidney adverse effects are high serum levels of lithium and longer time on lithium therapy[134]. Kidney biopsy samples from patients receiving lithium show diffuse tubulointerstitial nephritis[134] but whether this inflammation could cause dRTA is unclear and no studies have specifically examined intercalated cells. In a rat model of chronic lithium ingestion, increased pendrin expression and aberrant pendrin localization were observed in the inner medulla[135]. Hypothetically, increased pendrin activity could mediate inappropriate bicarbonate secretion into urine, resulting in renal acidosis similar to that seen in a mouse model of pseudohypoldosteronism type II (PHAII) with elevated pendrin activity[136]. Another study using a rat model demonstrated that lithium induced polyuria with more alkaline urine and increased urinary excretion of ammonium while the rats were mildly acidotic[137]. The researchers suggested that lithium might not cause dRTA but the combination of mild acidosis and more alkaline urine due to an increased ammonium buffer capacity might have led to misinterpretation of this state as dRTA. Further studies are needed to investigate the effect of lithium on renal acid excretion.

[H3] Antibiotics and antifungals. The antifungal amphotericin B has a range of nephrotoxic adverse effects including dRTA with normal anion gap[138]. Animal experiments and *in vitro* experiments with isolated perfused collecting ducts and turtle bladder suggest that amphotericin B may cause H^+-permeable pores that induce back-leak of H^+ from the tubular lumen into epithelial cells[139-141].

[H3] Potassium-sparing diuretics and mineralocorticoid receptor antagonists. Inhibition of collecting duct electrogenic Na^+-reabsorption by ENaC can cause dRTA, which is usually hyperkalemic due to impaired K^+-secretion and classified as type IV dRTA[2,142]. The potassium-sparing diuretics amiloride, benzamil and triamterene block ENaC and have been linked to this type of dRTA[143,144]. Likewise, mineralocorticoid receptor antagonist, such as canrenoate, spironolactone and eplerenone, reduce the stimulation of ENaC by aldosterone[145]. This effect is mimicked in patients with inactivating mutations in ENaC subunits[146].

[H3] Toluene. Toluene (also known as toluol) is an aromatic hydrocarbon that is manufactured as a solvent but also misused as an inhalant owing to its euphoric effects and easy accessibility. Toluene toxicity causes hypokalemic renal acidosis that can present clinically with muscular weakness, paralysis, confusion and abnormal ECG[147,148]. Toluene-induced acidosis can be with normal or elevated anion gap depending on the effects of toluene on the development of ketoacidosis or lactacidosis. A study of a small cohort of patients with toluene intoxication identified elevated levels of hippuric acid (a major metabolite of toluene) in plasma and urine with normal ammonium excretion and renal losses of sodium and potassium. The researchers suggested that toluene did not cause dRTA but the high hippuric acid levels resulted in an elevated anion-gap), a reduction in GFR due to volume contraction and urinary loss of potassium leading to hypokalemic acidosis[149]. Toluene is nephrotoxic and kidney biopsy samples can show diffuse damage to proximal and distal nephron segments [150]. The kidneys of newborns from mothers with toluene abuse may also be affected, mimicking inherited forms of dRTA[151].

Topiramate. The anti-migraine topiramate is a chemical derivative of the carbonic anhydrase II inhibitor acetazolamide and causes renal tubular acidosis due to the inhibition of carbonic anhydrases along the nephron [152]. Due to the important function of carbonic anhydrases in proximal tubule and intercalated cells, a mixed type of acidosis (type III) with features of proximal RTA and dRTA develops. Patients often develop kidney stones or nephrocalcinosis.

Vanadium. Vanadium (vanadate) is suspected to cause a form of endemic dRTA in northeastern Thailand[153,154]. The mechanism might involve inhibition of H^+K^+-ATPases in the collecting duct.

[H1] Clinical features of dRTA

Patients with autosomal recessive forms of dRTA typically present in the first year of life with growth failure or an acute illness, with blood tests revealing metabolic acidosis and hypokalemia. Occasionally, *ATP6V1B1*-dRTA is diagnosed later in childhood.[42,155,156]. Urine tests typically show an inappropriately alkaline pH and hypercalciuria. Most patients have polyuria with a urinary concentrating defect. Renal ultrasounds show nephrocalcinosis in almost all patients.[42,156] Additional evidence of a proximal tubulopathy, specifically low-molecular weight proteinuria, aminoaciduria and renal phosphate wasting is commonly seen at presentation and may initially suggest a diagnosis of renal Fanconi syndrome[155]. Glycosuria is usually absent. Correction of metabolic acidosis with alkali supplementation leads to resolution of proximal tubular symptoms, thus helping to establish the correct diagnosis. Children with autosomal dominant dRTA may be identified by family screening before overt symptoms become apparent or present later in childhood with growth failure or in adulthood with urolithiasis[156]. Rickets can be part of the initial presentation[157].

SLC4A1-dRTA can be either autosomal dominant[158] or autosomal recessive [59]. In autosomal dominant cases, the phenotype may be less severe than that of individuals with dRTA owing to mutations in *ATP6V1B1* or *ATP6V0a4*[159]. *SLC4A1*-dRTA often presents in adolescence or early adulthood, usually with recurrent calcium phosphate stone formation. Patients may have red cell deformities (spherocytosis or ovalocytosis)[160] that can improve with alkali therapy [161].

Inherited and acquired forms of dRTA are associated with renal and extrarenal features (**Figure 4**). Some of these features are direct consequences of the underlying defect, whereas others are caused by the disturbance of acid-base homeostasis. In general, patients with acquired forms of dRTA present with a combination of manifestations related to their underlying disease and dRTA. The age of onset of acquired dRTA is usually much later than for inherited dRTA and growth retardation is therefore not a problem. Also, salt wasting has not been reported for acquired dRTA while other electrolyte disorders such as hypokalemia can be more pronounced.

[H2] Renal manifestations

Several renal symptoms are frequently observed in patients with primary or acquired forms of dRTA.

[H3] Urinary acidification defect and acidosis. Reduced urinary acid excretion is a defining feature of dRTA. Patients with complete forms of dRTA present with normal anion-gap and hyperchloremic (and often hypokalemic) acidosis. Urine pH is inappropriately alkaline given the overt acidosis and most researchers use a threshold of urine pH >5.3 to diagnose dRTA[162,163]. Alkaline urine pH results from failure of acid-secretory type A intercalated cells to secrete protons into urine or more rarely from proton back-leak. Alkaline pH distinguishes classic type 1 dRTA and hyperkalemic type IV dRTA from proximal or mixed types of RTA in which urine pH can be more acidic. Patients with dRTA usually excrete reduced amounts of ammonium into urine. This reduction in ammonium excretion is at least partly due to a reduced pH gradient between the renal interstitium and the urine.

[H3] Hypercalciuria, hypocitraturia and renal calcifications. These features are caused by acidosis independent of the occurrence of dRTA and are often found in non-acidotic stone formers without evidence of dRTA. The combination of hypocitraturia and hypercalciuria, together with more alkaline urine, promotes the formation of calcium-phosphate and calcium-oxalate containing crystals and nephrocalcinosis or nephrolithiais. Stones in patients with dRTA are frequently composed of calcium phosphate. Thus, detection of calcium phosphate stones should prompt investigation for dRTA[164]. Urinary citrate excretion depends on the amount of citrate that is filtered by glomeruli and the rate of citrate reabsorption by Na^+/dicarboxylate cotransporter 1 (NaDC1, also known as SLC13A2) in the proximal tubule. Acidosis stimulates citrate reabsorption in the proximal tubule with consequent hypocitraturia[165]. Citrate usually complexes with calcium, increasing its solubility and reducing its availability to bind to oxalate or phosphate[166]. Hypercalciuria originates from increased bone resorption during acidosis and inhibition of renal calcium reabsorption[167]. Normalization of acid-base status also corrects hypercalciuria.

Nephrocalcinosis and nephrolithiasis are frequent in patients with primary and secondary forms of dRTA; approximately 65% show calcifications on plain X-ray[168]. In several cohorts of patients with primary dRTA, the prevalence of nephrocalcinosis

or nephrolithiasis was 90-100%[42,68,93]. Nephrolithiasis and nephrocalcinosis might contribute to the increased risk of CKD in patients with primary dRTA[169,170].

[H3] Proteinuria. Low molecular weight proteinuria is seen in some patients with dRTA and can be isolated or part of a more generalized proximal tubule dysfunction[93,171]. The symptoms mostly disappear with sufficient alkalinizing therapy[93].

[H3] Renal salt wasting. Some patients with inborn forms of dRTA experience renal salt wasting despite correction of acidosis[172]. Clinical observations in these patients suggested a defect in the collecting duct that was examined further in a mouse model that lacked the b1 H^+-ATPase subunit. This subunit is expressed in acid-secretory type A intercalated cells and in type B intercalated cells, which have a role in collecting duct salt reabsorption through the action of the luminal Cl-/HCO3- exchanger pendrin together with the electroneutral sodium bicarbonate exchanger 1 (NDCBE1, also known as SLC4A8). The actions of these exchangers lead to net NaCl reabsorption independent of the classic route mediated by ENaC in neighboring principal cells. In intercalated cells, H^+-ATPases energize transport processes by pumping protons either into urine or back into blood. In mice, disruption or lack of the b1 subunit of H^+-ATPases reduced pendrin expression and activity and caused renal salt wasting [22]. Moreover, absence of pendrin activity has been linked to decreased ENaC function and salt wasting in mice [173]. A similar defect in salt reabsorption would be expected with defective ATP6V0A4 as this subunit is also expressed in type B intercalated cells [79].

[H3] Hypokalemia. Hypokalemia is a frequent finding in dRTA and in severe cases can lead to muscle weakness or paralysis. Hypokalemia is likely caused by renal potassium losses while extracellular potassium levels are maintained for some time due to internal shifts of potassium from the intracellular space into the extracellular space in exchange for protons. Renal wasting of potassium might be partly driven by increased distal delivery of sodium and elevated aldosterone levels but the exact mechanisms remain elusive[174].

[H2] Extrarenal manifestations

All genes and proteins that are associated with primary dRTA have extrarenal expression: AE1 in red blood cells, B1 H^+-ATPase and A4 H^+-ATPase in inner ear, epididymis and pulmonary clear cells, FOXI1 in inner ear, epididymis and CFTR-rich

specific cells of the trachea and WDR72 in salivary glands, teeth, brain, lung and possibly in liver and thyroid. Thus, depending on the gene that is mutated, extrarenal symptoms may occur that are not caused by the direct effects of dRTA and are not easily ameliorated by dRTA therapies.

[H3] Inner ear. Patients with dRTA associated with *ATP6V1B1, ATP6V0A4* or *FOXI1* frequently experience progressive sensorineural hearing loss and deafness[68,70,90]. The hearing loss is not caused by systemic acidosis and consequently cannot be treated with alkali therapy. Most patients with ATP6V1B1-dRTA experience early onset of hearing deficits[68]. In patients with ATP6V0A4-dRTA, the onset, severity and prevalence of hearing deficits is more variable[68]. Only a few patients with FOXI1-dRTA have been reported and all had severe hearing deficits [90].

Loss of *ATP6V1B1, ATP6V0A4* or *FOXI1* is associated with sensorineural deafness with enlarged vestibular aqueduct (EVA) as detectable by CT. All three genes are highly expressed in mitochondria-rich marginal cells in the stria vascularis, which produces endolymph **[G]** [175]. These cells seem to be important for pH regulation of endolymph in the cochlear part of the inner ear. H^+-ATPases, including those with B1 and A4 subunits, secrete protons into endolymph, whereas a chloride-bicarbonate exchanger (AE1 or AE2) transports bicarbonate into intrastrial fluid. Loss of H^+-ATPase function alkalinizes cochlear endolymph. In the ear, H^+-ATPases are also found in interdental cells, cells lining the endolymphatic sac, inner hair cells and a subset of supporting cells in the organ of Corti[175].

Strikingly, the EVA phenotype resembles Pendred syndrome, which is caused by mutations in pendrin. Pendred syndrome is characterized by goiter and hypothyroidism and associated with sensorineural deafness. Pendrin is highly expressed in the luminal membrane of epithelial cells along the endolymphatic sac that also express H^+-ATPases at the luminal and/or basolateral side[176]. In these cells, H^+-ATPases and pendrin likely synergize in the reabsorption of chloride from the endolymph. Loss of function of either H^+-ATPases or pendrin might therefore lead to reduced salt and fluid absorption from endolymph, eventually causing EVA with increased pressure in the endolymph system[177]. Thus, H^+-ATPases may have a critical role in inner ear regulation of endolymph pH and volume.

Loss of FOXI1 in the inner ear reduces the transcription of target genes including pendrin, the A1, A4, B1 and E2 H^+-ATPase subunits and CAII, all of which are required for regulation of pH and fluid in the inner ear[91,98,178]. Notably, mice that were heterozygous for deletion of both Foxi1 and pendrin developed EVA, whereas mice that were heterozygous for either Foxi1 or pendrin variants did not, suggesting a gene-dosage effect on the development of inner ear pathology. In zebrafish, development of the otic vesicle is also under the control of FOXI1, which can determine the fate and formation of neuronal progenitor cells[179]. Thus, the pathology of inner ear disease is more complex in the case of defective *FOXI1* than for other dRTA genes because the defect will affect multiple pathways that are regulated by this transcription factor.

[H3] Erythrocytes. AE1 is a major constituent of the red blood cell membrane that mediates the release of HCO_3^- formed by intracellular CAII. This pathway is involved in peripheral removal and pulmonary exhalation of CO_2. However, no specific effect of *SLC4A1* mutations on ventilation and removal of CO_2 has been identified. AE1 also serves as an anchor for the cytoskeleton through binding of a protein complex that includes protein 4.2, spectrin, actin[180], glycophorin A, Rh-associated glycoprotein (RHAG) and glycolytic enzymes that regulate red blood cell metabolism and survival[180,181]. *SLC4A1* mutations that cause SAO are frequent in countries with a high prevalence of *Plasmodium falciparum* infections and seem to confer strong resistance against cerebral malaria[182]. SAO variants confer a large erythrocyte cation leak[183] much like the autosomal recessive dRTA-causing variants that are found exclusively in malaria endemic regions[184].

[H3] Epididymis. ATP6V0A4 and ATP6V1B1 are found in proton-secreting clear cells in the epididymis that acidify epididymal fluid to immobilize sperm and enable its maturation[185]. Mouse models that were deficient for either subunit did not show evidence of male infertility[186,187]. No data are available on fertility in patients with dRTA.

[H3] Olfactory cells. H^+-ATPases are also found in sustentacular cells in the olfactory epithelium. Mice that were deficient in Atp6v1b1 or Atp6v0a4 showed evidence of reduced olfactory function, suggesting hypoosmia[187,188]. Sense of smell has not been examined in patients with dRTA.

[H3] Teeth. Patients with mutations in WDR72 have amelogenesis imperfecta. WDR72 seems to be involved in trafficking of calcium transporters and vesicles containing calcium for mineralization[189].

[H3] Bone. Bone contains mostly calcium apatite consisting of calcium, hydroxyl ions and phosphate, which is an important source of buffers in chronic acidosis. During acidosis, protons can either directly react with apatite, leading to chemical bone dissolution, or stimulate osteoclasts and inhibit osteoblasts, leading to enhanced bone resorption[190,191]. Low extracellular pH may be sensed by the proton-activated receptor ovarian G-protein coupled receptor 1 (OGR1, also known as GPR68) activating osteoclasts, but the physiological relevance of this regulation is not fully understood [192,193]. Furthermore, acidosis may stimulate parathyroid hormone (PTH) secretion and reduce calcitriol synthesis, thereby further stimulating osteoclast activity[194-196]. Collectively, the effects of acidosis on bone result in reduced mineralization, altered bone remodeling, reduced trabecular bone mineral density, lower trabecular volume, and ultimately reduced bone stability.

Failure to thrive is seen in up to 80% of patients with primary dRTA and involves poor skeletal growth[42,69,70]. Adults with primary dRTA may have reduced stature independent of the underlying genetic cause[68-70]. On plain X-ray, typical findings in children with dRTA include bowlegs, an altered epiphysis-metaphysis zone with cupping and fraying and Looser zones, indicating vitamin insufficiency and fractures. Importantly, bone symptoms resolve with appropriate alkali therapy in children and adults[197].

[H2] Treatment

dRTA is a treatable disease and virtually all symptoms, except deafness, resolve with appropriate alkali supplementation. In response to this treatment, biochemistries normalize and patients demonstrate increased activity and appetite with catch-up growth. This resolution is consistent with the important role of acid-base homeostasis in normal physiology, including growth and development[198]. However, nephrocalcinosis is typically persistent, while hypercalciuria resolves.[156] Alkali doses of 2-4mEq/kg/day are usually used for treatment of dRTA but some patients are prescribed as much as 10 mEq/kg/day, with younger children generally receiving higher doses, likely reflecting their increased metabolic rate and consequently

increased acid load as well as high bone formation[156]. Adequate treatment seems to be challenging. In one large retrospective study involving 340 patients with a clinical diagnosis of dRTA, only half achieved adequate metabolic control, as measured by normalization of plasma bicarbonate and urine calcium. Importantly, adequate metabolic control was associated with increased final height and higher eGFR at last follow-up[156]. In this study, a third of children and more than 80% of adults with dRTA had an eGFR <90 ml/min/1.73m^2 (CKD stage ≥2) at last follow-up. The aetiology of low eGFR is unclear, but is consistent with CKD observed in other cohorts of patients with dRTA[69,70] or other tubulopathies[199].

A variety of different alkali salts, typically containing bicarbonate or citrate, are used to treat dRTA, depending on local availability. Three to four times daily administration is usually prescribed to maintain acid-base homeostasis. However, a microgranular preparation of potassium-bicarbonate and potassium-citrate that requires only twice daily administration has been developed[200]. Dietary approaches to reduce intake of sodium and acid-releasing animal proteins may help to reduce acidosis[201] and hypercalciuria. Thiazide diuretics may also help to reduce hypercalciuria[202] and increase urine volume to reduce the risk of stone formation.

[H1] Incomplete dRTA

dRTA without overt systemic acidosis, termed incomplete dRTA, was first reported more than 60 years ago in a study that used a urine acidification test with oral ammonium chloride to detect impaired acid excretion in individuals with and without kidney disease[163]. In this study, three patients had medullary nephrocalcinosis and a urine pH >5.3 but no systemic metabolic acidosis. However, similar to patients with dRTA, they failed to acidify their urine to pH <5.3 after administration of ammonium chloride but did show increases in urinary ammonium excretion and titratable acidity. The increase in urinary ammonium and titratable acidity may explain why these patients do not develop overt acidosis under baseline conditions.

dRTA occurs in a substantial subset of patients with and without kidney stone disease. However, data from multiple studies have highlighted a close relationship between stone formation and incomplete dRTA[203,204]. Determining the prevalence of incomplete dRTA is challenging because the lack of acidosis in these patients makes their alkaline

urine non-diagnostic, necessitating a urinary acidification test,[205,206] and accurate epidemiological data are lacking. Nevertheless, data on stone-forming patients screened for incomplete dRTA using urinary acidification tests suggest a prevalence in this population of 2-19%[203,205,207,208].

The absence of systemic acidosis in patients with incomplete dRTA despite a urinary acidification defect that is functionally no different from that of patients with complete dRTA is poorly understood. A potential explanation is buffering of non-secreted protons by phosphate liberated from the skeleton. Indeed, children with incomplete dRTA have reduced growth[209], which can be reversed by treatment with bicarbonate[210]. Furthermore, a prevalence of incomplete dRTA of 19-22% was reported in studies of patients with 'primary osteoporosis' (i.e., unexplained low bone mineral density or vertebral fractures)[211,212]. However, a community study of healthy adults in North-East Thailand reported no significant difference in bone mineral density between individuals with incomplete dRTA and those with no acidification defect [213].

If skeletal reabsorption of phosphate was the only factor that prevented acidosis in incomplete dRTA, one would expect an increase in the urinary titratable acidity as compared to people without dRTA, which is a measure of the urinary buffered hydrogen ions with the main buffer being phosphate[214]. However, in a very small series of patients with incomplete dRTA receiving the oral ammonium chloride test, titratable acidity seemed to be reduced with no compensatory increase in ammonium excretion[215]

Incomplete dRTA might be caused by any cause of primary or acquired dRTA and could potentially be considered a pre-acidotic form of the complete syndrome[163]. Case reports exist of children with pathogenic mutations in *SLC4A1* who showed incomplete RTA during their first years of life before developing systemic acidosis[155,216]. Observations in a single family also suggest that heterozygous carriers of pathogenic variants in *ATP6V1B1* can show clinical evidence of incomplete dRTA[217]. In two cohorts of stone formers, a polymorphism in *ATP6V1B1* resulting in the missense variant p.E161K was associated with reduced urinary acidification following the ammonium chloride test and more frequent calcium phosphate-containing stones [218]. This finding is consistent with observations in heterozygous Atp6v1b1-knockout mice[219]. Further studies are needed to investigate the genetic basis of incomplete

dRTA. Use of exome or whole genome sequencing in combination with careful clinical phenotyping of patients may be informative.

Incomplete dRTA has also been described in patients with medullary sponge kidney[220], Sjögren syndrome [221], nephrocalcinosis (including hereditary forms[222]) and drug toxicity.

Similar to complete dRTA, typical stone composition in incomplete dRTA is of calcium phosphate (stones may be >95% carbonate apatite)[223]. The alkaline urine favors the precipitation of calcium phosphate and thereby increases the risk of kidney stones and nephrocalcinosis. Incomplete dRTA is frequently associated with hypocitraturia but only variably associated with hypercalciuria[204].

[H2] Diagnosis

The gold standard method for diagnosis of incomplete dRTA is still considered to be urine acidification with oral administration of 0.1g/kg of ammonium chloride (NH_4Cl), known as the short ammonium chloride test.[163]. This test has a high rate of gastrointestinal adverse effects, mainly nausea and vomiting. Alternative diagnostic methods have been suggested, including the simultaneous F+F test, which uses 40mg of furosemide and 1mg of fludrocortisone to activate collecting duct ENaC and increase sodium chloride delivery to the collecting duct to promote proton secretion. However, the F+F test might also stimulate thick ascending limb H^+-secretion by sodium/hydrogen exchanger 3 (NHE3) and is not a measure of connecting tubule and cortical collecting duct function[224]. The F+F test does not cause gastric irritation and stimulates urinary acidification similar to ammonium chloride[225]. In stone forming patients, the F+F test is reported to have a sensitivity of 85% and a specificity of 77%, compared to the short ammonium chloride test[205]. A morning urine threshold of pH <5.3 usually excludes the presence of incomplete dRTA[205].

[H2] Treatment

The treatment of patients with incomplete dRTA and recurrent stone disease is based on alkali supplementation[206]. Due to the rarity of the diagnosis, no randomized controlled trials have assessed the effect of alkali therapy on stone or bone disease in incomplete dRTA. However, data from some small studies exist. Citrate therapy was shown to reduce stone recurrence and improve bone health, hypercalciuria and

citraturia in 9 patients [226]. A longitudinal study in 40 children with complete or incomplete dRTA reported that oral alkali therapy resulted in significant increases in height standard deviation scores compared with healthy children[210]. Potassium citrate is the most commonly recommended therapy but sodium bicarbonate is also widely used in clinical practice. Sodium-based salts are avoided by some physicians owing to a theoretical risk of increased calciuria; however, this risk seems to correlate more closely with systemic acidosis than with sodium supplementation[227].

[H1] Conclusions

dRTA is a tubulopathy that affects multiple organ systems either because of defects in genes that share expression between kidney and other organs or because acidosis affects extrarenal systems. Primary forms often manifest early in life, while acquired forms typically occur in the 4th to 6th decade and are caused by autoimmune disease or adverse effects of commonly used drugs. Early recognition and diagnosis of primary forms of dRTA is important to prevent failure to thrive and to identify children with forms that are associated with sensorineural hearing impairment who may require hearing aids and special attention at school. Primary dRTA is associated with an increased risk of developing CKD, whereas dRTA secondary to autoimmune disease or drug use often occurs on a background of impaired kidney function. Alkalinizing therapies can prevent most of the symptoms of dRTA that are related to acidosis but has no impact on loss of hearing. Whether alkalinizing therapy can prevent or delay loss of kidney function in primary dRTA remains to be firmly established. Incomplete dRTA is found in a subset of patients with recurrent kidney stone disease and may be a continuum of primary dRTA. This form of dRTA may be more common than primary dRTA but is often not detected owing to the need for provocation tests for diagnosis. The genetic basis of incomplete dRTA requires further study. In the future, increased understanding of this disease may facilitate improved diagnosis.

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Author contributions

C.A.W., S.C.L.-G., D.B. and S.W. researched data for the article. C.A.W., S.C.L.-G. and S.W. wrote the article. All authors contributed substantially to discussion of the content and reviewed or edited the manuscript before submission.

Competing interests

C.A.W. reports honoraria from Advicenne, Kyowa Kirin, Chugai, and Medice/Salmon Pharma. DB has received honoraria from Advicenne. R.U. is currently employed by AstraZeneca BioPharmaceuticals R&D, Early CVRM (CardioVascular Renal and Metabolism), Cambridge UK. The other authors declare no competing interests.

Peer review information

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Key Points

- Primary distal renal tubular acidosis (dRTA) is caused by pathogenic variants in at least 5 different genes: *SLC4A1*, *ATP6V0A4*, *ATP6V1B1*, *FOXI1* and *WDR72*; additional unidentified genes might also contribute
- Acquired forms of dRTA are often found in patients who have autoimmune disorders or who take drugs that reduce the ability of the kidney to excrete acids
- Although kidney pathologies in dRTA are mostly restricted to intercalated cells, systemic acidosis also affects other renal cell types and extrarenal organs
- Pathogenic variants in all known dRTA genes also cause extrarenal pathologies due to their expression in the inner ear, red blood cells or teeth.
- Primary and secondary forms of dRTA should be diagnosed and treated to prevent the sequelae of systemic acidosis on growth and bone stability; primary dRTA might also be a risk factor for the development of chronic kidney disease. Incomplete dRTA is often associated with kidney stone disease and may represent an intermediate pre-acidotic form of the complete syndrome

Table 1: Genes that are mutated in patients with primary dRTA

Gene	Protein	Function	Inheritance	OMIM*
SLC4A1	Anion exchange protein 1 (AE1)	Cl^-/HCO_3^- anion exchanger	AD or AR	#611590
ATP6V1B1	V-type proton ATPase subunit B, kidney isoform	H^+-ATPase subunit	AR	#267300
ATP6V0A4	V-type proton ATPase 116 kDa subunit a4	H^+-ATPase subunit	AR	#602722
FOXI1	Forkhead box I1 (FOXI1)	Transcription factor	AR	#600791‡
WDR72	WD repeat-containing protein 72 (WDR72)	Unknown	AR	#613211

*Online Mendelian Inheritance in Man: https://www.omim.org/. ‡No distinct OMIM number has been assigned to FOXI1 mutations, the number refers to a phenotype. AD, autosomal dominant; AR, autosomal recessive; dRTAm, distal renal tubular acidosis.

Table 2: Autoimmune diseases that are associated with dRTA

Disease	Main symptoms	Patho-mechanism of dRTA	Refs
Sjögren's syndrome	Dry eyes and mouth (sicca syndrome), interstitial nephritis	Loss of intercalated cells possibly owing to autoantibodies	
Rheumatoid arthritis	Fatigue, fever, pain and swelling of small joints	Unknown	
Systemic lupus erythematosus	Fatigue, skin rashes, fevers, pain or swelling in joints, may affect heart, kidneys and brain	Unknown	
Primary biliary sclerosing cirrhosis	Liver disease, bone and joint pain, fatigue	Loss of intercalated cells possibly owing to autoantibodies?	

dRTA, distal renal tubular acidosis.

Table 3: Drugs that are associated with dRTA

Drug	**Target or mechanism**	**Refs**
Lithium	Increased ammonium excretion and pendrin expression	135,137
Amphotericin B	H^+-back leak into epithelial cells	138,141
Toluene	H^+-secretion	228,229
Amiloride, benzamil, triamterene	Block ENAC, amiloride also blocks NHE3 at higher doses	143
Trimethoprim	Blocks epithelial Na^+ channel	230
Vanadium	Might block ATPases	153
Anti-migraine (e.g. topiramate)	Inhibits carbonic anhydrase II and IV	152
Calcineurin inhibitors	Calcineurin inhibitors may upregulate pendrin causing excessive bicarbonate secretion	231-233

dRTA, distal renal tubular acidosis; ENaC, epithelial Na+ channel; NHE3, sodium/hydrogen exchanger 3.

Figure 1 | Repertoire of cells in the collecting duct system. a | Schematic diagram depicting different types of intercalated cells and principal cells in the connecting tubule and cortical collecting duct. Type A intercalated cells express basolaterally kAE1 and apically H^+-ATPases and are the main acid excretory cells while type B intercalated cells have apically pendrin and basolateral H+-ATPases resulting in net bicarbonate secretion. Non-A/non-B intercalated cells also express pendrin and H+-ATPases but the exact role is not resolved. Principal cells express the ENaC and ROMK channels to reabsorb sodium and secrete potassium. Water is reabsorbed by the AQP2 and AQP3 water channels. In vivo intercalated cells are interspersed between principal cells. **b** | Human kidney biopsy sample showing type A intercalated cells stained for AE1 (green) and the B1 H^+-ATPase subunit (red), nuclei in blue. **c** | Human outer medullary collecting duct stained for a4 H^+-ATPases (red) and the principal cell specific water channel aquaporin 2 (AQP2, green). **d** | Human cortical collecting duct stained for pendrin (green) and B1 H^+-ATPase (red). AE1: anion exchanger 1 (SLC4A1), CA II: carbonic anhydrase II, RhCG: rhesus protein RhCG, RhBG /RhCG: rhesus proteins RhBG and RhCG, Pds: pendrin (SLC26A4), AE4: anion exchanger 4 (SLC4A9), NDBCE: Na+-dependent chloride-bicarbonate exchanger (SLC4A8), ENaC. Epithelial Na^+-channel, ROMK: renal outer medullary K^+-channel, TA: titratable acidity.

Figure 2: Role of the transcription factor FOXI1 in intercalated cell differentiation. Mature intercalated cells and principal cells are formed from AQP2 expressing precursor cells (AQP2+). Secretion of the NOTCH1/2 ligand Jag1 activates NOTCH1/2 via a mechanism that might involve the proteases ADAM10 and γ-secretase. Active NOTCH forms a complex with the DNA-binding protein RBPJ and the resulting signaling suppresses Jag1 and activates ETS-related transcription factor ELF5 (ELF5), the histone-lysine N-methyltransferase DOT1L and the transcription factor HES1, leading to the expression of principal cell genes such as the epithelial sodium channel (ENaC) and AQP2 and suppressing the transcription factor forkhead box protein I1 (FOXI1). By contrast, differentiation into the intercalated cell lineage requires suppression of NOTCH1/2 signaling and secretion of Jag1. The E3 ubiquitin-protein ligase MIB1, transcription factor CP2-like protein 1 (TFCP2L1) and FOXI1

promote Jag1 secretion. FOXI1 activity is enhanced by TFCP2L1 and drives the expression of typical intercalated cell genes such as kidney anion exchange protein 1 (kAE1), pendrin, carbonic anhydrase II (CAII) and various subunits of the H+-ATPase. Absence of functional FOXI1 causes loss of differentiation and the appearance of a cell type that expresses CAII together with AQP2 and other principal cell proteins.

Figure 3: Cellular pathophysiology of dRTA-causing mutations in *SLC4A1, ATP6V1B1, ATP6V0A4* and *WDR72*. a| Impact of mutations in *SLC4A1*, which encodes anion exchange protein 1 (AE1). After synthesis and maturation in the endoplasmic reticulum (ER) and Golgi apparatus, wild-type kidney AE1 (kAE1) is trafficked to the basolateral membrane of type A intercalated cells. Mutant forms of kAE1 are either retained in the ER or Golgi then degraded in endosomes and lysosomes, mistargeted to the apical membrane or inserted into the basolateral membrane but rapidly degraded owing to decreased stability. **b** | Kidney biopsy samples from normal kidney and from a patient with dRTA owing to a heterozygous *SLC4A1* mutation (S613F). Normal kidney stained for AE1 (green) and AQP2 water channel (red). In the sample from the patient, most cells are stained for AQP2, whereas AE1 staining is seen in red blood cells but not in intercalated cells. **C** | Impact of mutations in *ATP6V1B1* and *ATP6V0A4*, which encode the ATP6V1B1 (B1) and ATP6V0A4 (A4) H^+ATPase subunits, respectively, and in WDR72, which encodes WD repeat-containing protein 72 (WDR72). In type A intercalated cells, assembly and trafficking of H+ ATPase pumps containing wild type A4 and B1 subunits to the apical membrane is enhanced by acidosis or angiotensin II. Pumps that contain mutant A4 (mtA4), mutant B1 (mtB1) or wild type B2 instead of mtB1 have reduced assembly and trafficking and are unable to respond to acidosis or angiotensin II. WDR72 is thought to be involved in vesicular trafficking and/or assembly of pumps but its exact function remains to be established. The loss of function of mutant WDR72 (mtWDR72) may reduce insertion of intact pumps into the apical membrane. AT1R, type-1 angiotensin II receptor.

Figure 4: Spectrum of symptoms associated with primary dRTA. Direct symptoms of distal renal tubular acidosis (dRTA) are caused by cellular defects in organs expressing dRTA-related genes including the kidney, ear, and teeth, whereas indirect

symptoms including nephrolithiasis are mostly due to acidosis and are usually improved by alkalinizing therapy.

Fig 1

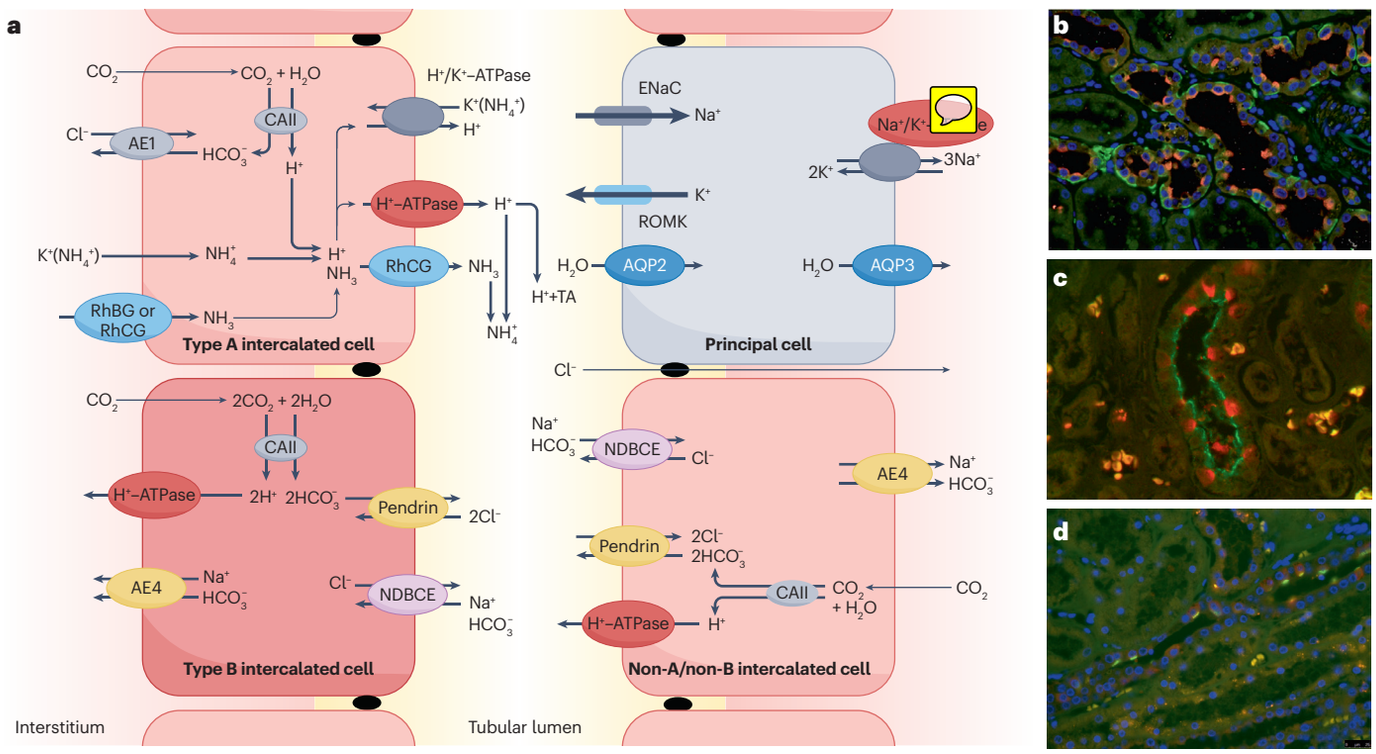

a
CO2
CO2 + H2O
CAII
Cl−
AE1
HCO3−
H+
H+/K+–ATPase
K+(NH4+)
H+
H+–ATPase
H+
K+(NH4+)
NH4+
H+
NH3
RhCG
NH3
RhBG or RhCG
NH3
Type A intercalated cell
H+TA
NH4+
ENaC
Na+
Na+/K+
2K+
3Na+
K+
ROMK
H2O
AQP2
H2O
AQP3
Principal cell
Cl−
CO2
2CO2 + 2H2O
CAII
H+–ATPase
2H+
2HCO3−
Pendrin
2Cl−
AE4
Na+
HCO3−
Cl−
NDBCE
Na+
HCO3−
Type B intercalated cell
Na+
HCO3−
NDBCE
Cl−
AE4
Na+
HCO3−
Pendrin
2Cl−
2HCO3−
CAII
CO2
+ H2O
CO2
H+–ATPase
H+
Non-A/non-B intercalated cell
Interstitium
Tubular lumen
b
c
d

Fig 2

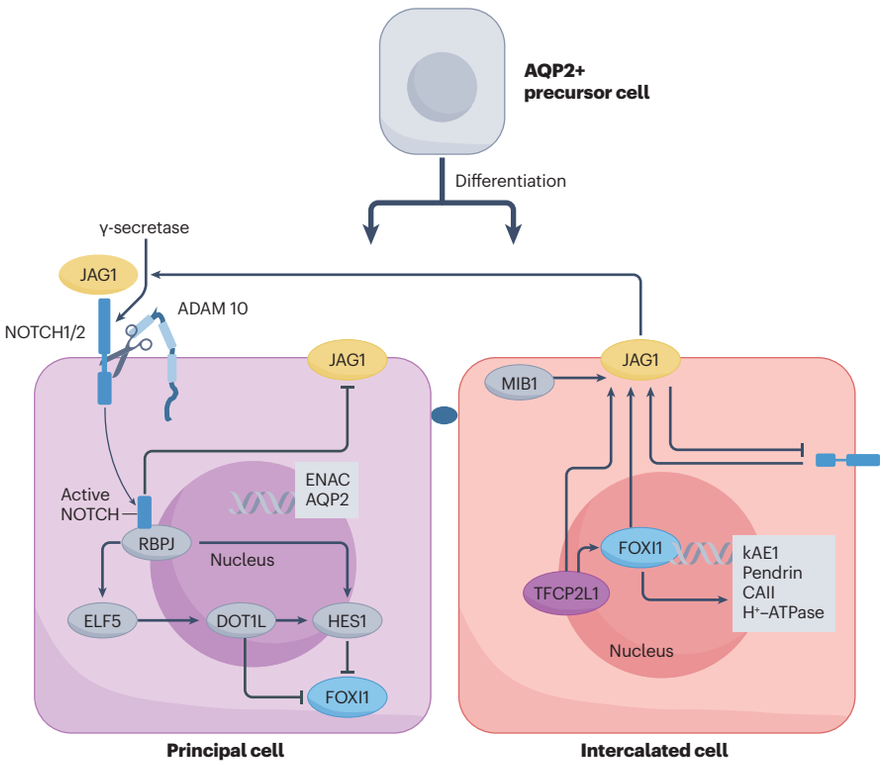

AQP2+
precursor cell
Differentiation
γ-secretase
JAG1
ADAM 10
NOTCH1/2
JAG1
Active
NOTCH
ENAC
AQP2
RBPJ
Nucleus
ELF5
DOT1L
HES1
FOXI1
Principal cell
JAG1
MIB1
FOXI1
kAE1
Pendrin
CAII
H⁺–ATPase
TFCP2L1
Nucleus
Intercalated cell

Fig 3

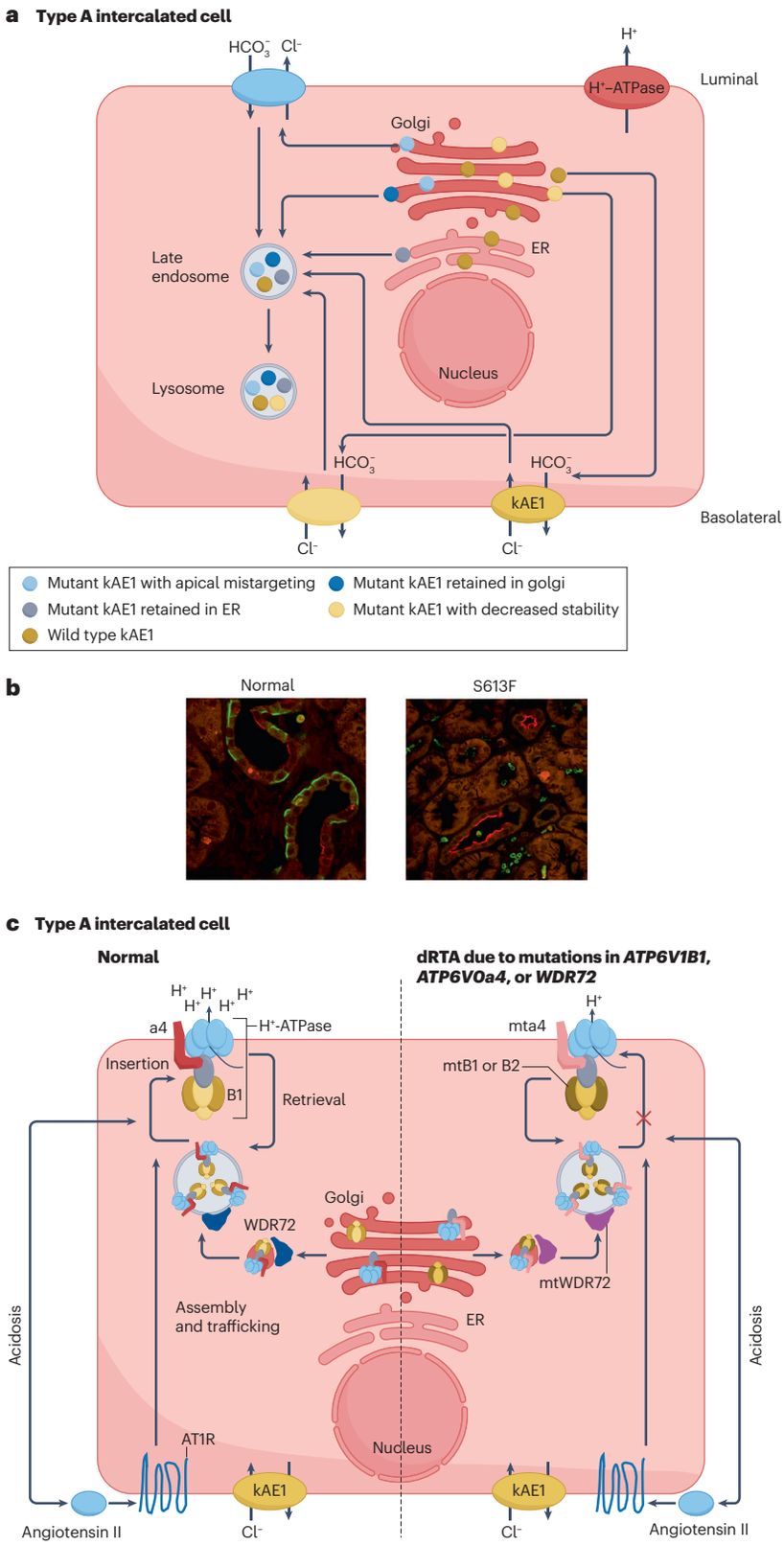
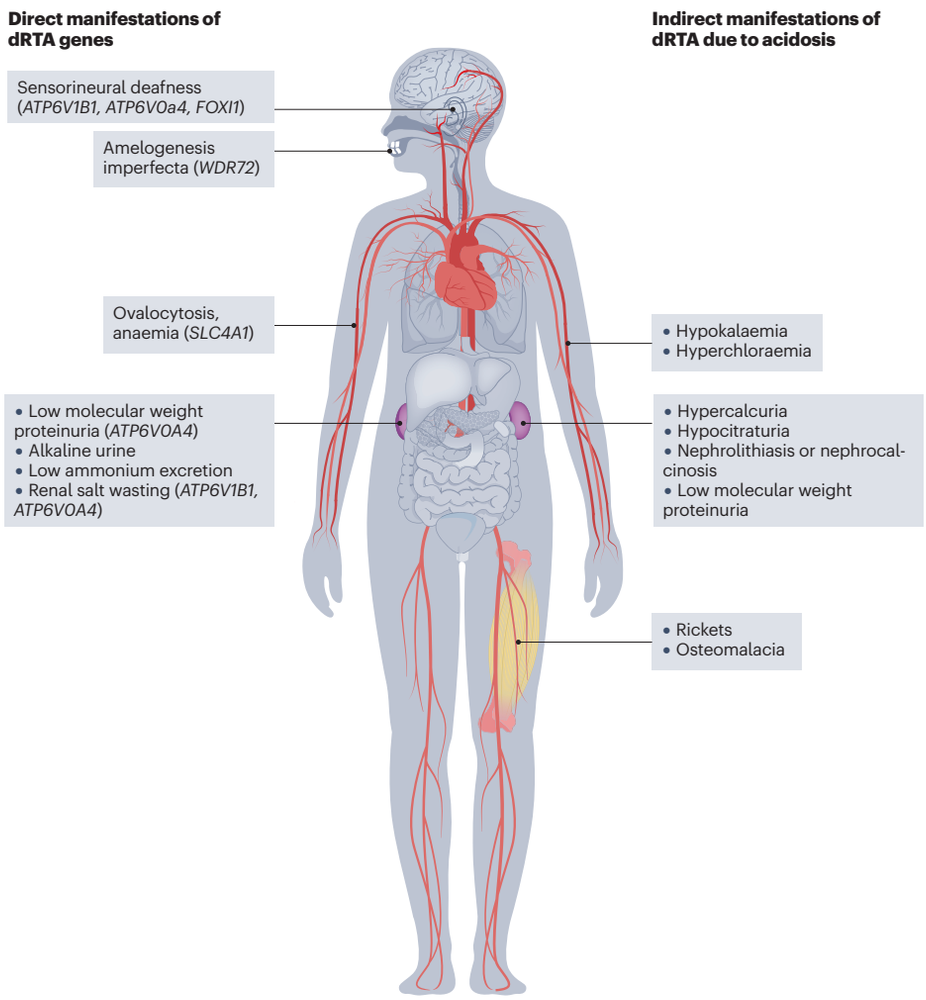

a Type A intercalated cell
HCO3- Cl-
H+
H+–ATPase
Luminal
Golgi
ER
Late endosome
Nucleus
Lysosome
HCO3-
HCO3-
kAE1
Basolateral
Cl-
Cl-
Mutant kAE1 with apical mistargeting
Mutant kAE1 retained in golgi
Mutant kAE1 retained in ER
Mutant kAE1 with decreased stability
Wild type kAE1
b
Normal
S613F
c Type A intercalated cell
Normal
dRTA due to mutations in ATP6V1B1, ATP6V0a4, or WDR72
H+
a4
H+-ATPase
mta4
Insertion
B1
Retrieval
mtB1 or B2
Golgi
WDR72
mtWDR72
Assembly and trafficking
ER
Acidosis
Acidosis
AT1R
Nucleus
kAE1
kAE1
Angiotensin II
Cl-
Cl-
Angiotensin II

Fig 4


Direct manifestations of dRTA genes
Indirect manifestations of dRTA due to acidosis
Sensorineural deafness (ATP6V1B1, ATP6V0a4, FOXI1)
Amelogenesis imperfecta (WDR72)
Ovalocytosis, anaemia (SLC4A1)
• Low molecular weight proteinuria (ATP6V0A4)
• Alkaline urine
• Low ammonium excretion
• Renal salt wasting (ATP6V1B1, ATP6V0A4)
• Hypokalaemia
• Hyperchloraemia
• Hypercalcuria
• Hypocitraturia
• Nephrolithiasis or nephrocalcinosis
• Low molecular weight proteinuria
• Rickets
• Osteomalacia