Inherited Distal Renal Tubular Acidosis

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Renal acid-base balance may become deranged in a number of ways, some of which are the consequence of inherited disorders. Two main groups of distal renal acidopathies result either from a direct inability to secrete acid in the distal nephron (giving rise to type 1 renal tubular acidosis [RTA]) or as an inherited or functional consequence of hypoaldosteronism, leading to type 4 (hyperkalemic) RTA. In this review, I will discuss one category of the expanding class of inherited renal tubular transport disorders: the primary distal (type 1) RTAs. Though they are relatively rare in Western populations, they occur more commonly in areas of the world where rates of parental consanguinity are high. This has facilitated genetic studies, and the insights gained in understanding their molecular bases have added significantly to our elucidation of normal human physiology. The hope is that these findings may in the future prove relevant to the understanding and treatment of more common disorders and provide new therapeutic avenues.

Intercalated Cells

Many studies, mainly in rodents, have described two types of intercalated cells (IC): α and β. α-IC are responsible for coupled apical secretion of protons into the urine and reclamation of bicarbonate across the basolateral surface (Figure 1). On the apical surface, the multi-subunit proton pump (H^+-ATPase) transfers H^+ into the urine. It is of the same type as the H^+-ATPase found ubiquitously in intracellular organelles, such as lysosomes, which ensure suitably low pH for efficient function (1). H^+-ATPase are composed of at least 13 different subunits, organized into a membrane-anchored V_0 (stalk) domain through which protons are moved and a V_1 head that hydrolyzes ATP. The source of this ATP may well be glycolytic rather than mitochondrial, as evidenced by the recent finding of a physical interaction between the E subunit and aldolase, one of the glycolytic enzymes (2). At least two of the α-IC apical pump's subunits, the B subunit in the V_1 domain and the a subunit in the V_0 domain, have been found to be different, tissue-specific isoforms (*v.i.*). Thus, apical renal pumps contain B1 rather than B2 subunits (3), and a4 instead

of a1 (4). Being ubiquitously expressed, B2 and a1 may be regarded as housekeeping isoforms. Apical H^+-ATPase function, which is inhibitable by bafilomycin (5), is functionally coupled to basolateral bicarbonate exit (in exchange for chloride) *via* the anion exchanger AE1, described in more detail below.

β-IC essentially reverse this process. There remains a lack of consensus as to whether α- and β-IC are molecular mirror images of each other or separate cell types. Apparent plasticity of molecular targeting according to ambient pH and/or acid and alkali load *in vivo* (6) or epithelial cell seeding density *in vitro* (7) supports the former theory, but molecular analysis does not (8). One reason for this disparity is that immunolocalization studies of AE1 that demonstrate basolateral staining of α-IC have failed to demonstrate the molecule apically in β-IC. Whether this is because of epitope masking of the latter is not known. Two other potential Cl^-/HCO_3^- exchangers, pendrin and AE4, have been reported and may reside apically in β-IC (9,10). Defects in pendrin cause Pendred syndrome of deafness and goitre, but alkalosis is not a feature in either Pendred patients or pendrin knockout mice (9). AE4 has been described apically in β-IC of the rabbit (11) but not yet localized within the human kidney. In any event, the acid load provided by an omnivorous human diet dictates that the majority of IC will be acid-secretory (α), and molecular defects causing human inherited dRTA appear to date to be confined to this cell type.

Animal studies have identified additional P-type ATPase present apically in α-IC, which exchange protons for K^+ (12). In humans, however, the overall contribution of H^+/K^+-ATPase to α-IC function is not clear.
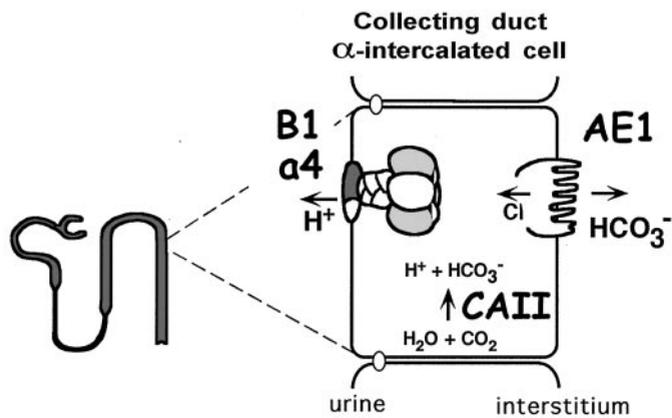
Distal RTA

The cellular basis of almost all human distal RTA is that α-IC fail to perform their normal function. This hypofunction most commonly arises as a secondary phenomenon; for example, in the context of drugs or hypo-aldosteronism, when it is referred to as type 4 RTA. In this context, it is usually accompanied by hyperkalemia. In contrast, the term dRTA is usually reserved for that associated with hypokalemia, (type 1 RTA), which most commonly appears in the setting of autoimmune disease, other systemic disorders, drugs, or as an intrinsic defect of the α-IC.

At the time of early descriptions, dRTA was thought to be due to back-leak of normally secreted protons across a leaky tubular epithelium. Over the following five decades, it became evident that acid secretion itself is abnormal in most cases, resulting from failure of hydrogen ion secretion. Until recently,

Figure 1. Molecular contributors to inherited dRTA. A proton pump is depicted schematically at the apical α-IC surface. *ATP6V1B1* and *ATP6V0A4* encode the B1 (light gray) and a4 (dark gray) subunits, respectively. *SLC4A1* encodes the heterotopic Cl^-/HCO_3^- exchanger, drawn on the basolateral side. Substrate for both transporters is provided by the catalytic activity of CAII, which is also present in the proximal tubule and osteoclast.

the molecular pathophysiology remained somewhat elusive. It is now clear that defective function in at least three components of the α-intercalated cell's polarized membrane transporters can cause primary dRTA.

Primary dRTA arises when the collecting duct fails to remove excess acid into the urine. It is therefore biochemically characterized by failure of the kidney to produce acid urine appropriately in the presence of systemic metabolic acidosis in the setting of otherwise normal renal function and was first recognized nearly 70 yr ago (13,14). Traditionally, if the metabolic acidosis is compensated such that arterial pH is within normal limits, dRTA can be diagnosed by demonstrating failure of urinary acidification after oral ammonium chloride administration. In view of the unpalatability of this compound, some investigators substitute furosemide (15), because increased distal sodium delivery should also result in urine acidification (and kaliuresis) under normal circumstances. Walter *et al.* further refined this by the prior addition of fludrocortisone to provide a mineralocorticoid load. In normal subjects, they showed tight correlation with the results of ammonium chloride loading that was not observed with furosemide alone (16).

Failure to appropriately excrete the normal nonvolatile acid products of the diet in dRTA results in hyperchloremic metabolic acidosis of varying severity. Primary dRTA is almost always accompanied by variably severe nephrocalcinosis and/or nephrolithiasis associated with hypercalciuria. Urinary citrate is low in dRTA because citrate reabsorption is upregu-

lated in the proximal tubule to provide new bicarbonate (1 citrate = 2 bicarbonate). Abnormal calcium deposition in dRTA is attributed in large part both to this hypocitraturia and to urine alkalinity, but the exact mechanisms for and precise sites of this deposition are unclear. It is also unclear why some patients present with stones but no overt nephrocalcinosis while others display the opposite. Serum calcium and phosphate levels are normal. The chronic acidosis and low bicarbonate result in obligate leaching of bone, resulting in rickets or osteomalacia in severe or untreated cases. The biochemical picture in primary dRTA usually includes hypokalemia, but again the precise mechanism is unclear. The renin/aldosterone axis is activated in dRTA, and sodium reabsorption by more proximal segments is probably impaired; it is therefore accepted that the consequent principal cell upregulation plays a part. In addition, increased K^+ secretion may be a mechanism for maintenance of electrical equilibrium.

Dominant and Recessive dRTA

Both autosomal dominant and autosomal recessive inheritance patterns have been reported in primary dRTA. The spectrum of clinical severity is very wide, ranging from compensated mild acidosis, absence of symptoms, and the incidental finding of stones and/or renal tract calcification to major effects in infancy with severe acidosis, impaired growth, and early nephrocalcinosis causing eventual renal insufficiency (Table 1). In general, though not invariably, patients with dominant (type 1a) dRTA display a milder phenotype than do those with recessively inherited disease. However, growth impairment and rickets may be evident in dominant disease. Erythrocytosis has been noted among patients with dominant disease (17). In addition, among patients with recessive but not dominant dRTA, a substantial fraction has progressive and irreversible bilateral sensorineural hearing loss (SNHL; dRTA type 1b) (18). A recent report describes radiologic vestibular aqueductal widening in association with recessive dRTA (19), but this abnormality is not pathognomonic, also being seen in isolation and in other syndromes such as Pendred and Branchio-Oto-Renal syndrome (20,21).

Autosomal Dominant dRTA (Type 1a; MIM #179800)

Three groups have identified ten dominant dRTA kindreds in which affected individuals are heterozygous for mutations in *SLC4A1* (22–24). This represents the first molecular evidence that anion rather than cation transport function is the major defect in dominant dRTA, and there is to date no evidence for genetic heterogeneity.

In the kidney, AE1 (kAE1) at the basolateral surface of α-intercalated cells is an 846 amino acid protein with multiple transmembrane spans (whether 12 or 14 has not yet been completely resolved) (25,26). It is encoded by a gene present on chromosome 17, and this same gene gives rise to an N-terminally extended 911 amino acid isoform with a separate promoter, expression of which is confined to erythrocytes (eAE1) (27). Much of the structural and functional work on AE1 has been concerned with eAE1, which, unlike kAE1,

Table 1. Clinical and biochemical features of primary renal tubular acidosis (RTA) affecting the distal nephron

	Dominant dRTA	Recessive dRTA	Osteopetrosis with RTA
Usual presentation	Older/adult	Infancy/early childhood	Childhood
Symptoms/signs	None?	Early nephrocalcinosis	Early nephrocalcinosis
	Nephrolithiasis	Vomiting/dehydration	Thickened bones
	Nephrocalcinosis	Poor growth	Cerebral calcification
	Sometimes rickets	Rickets	Mental retardation
	Sometimes osteomalacia	Bilateral SNHL in about 1/3, usually severe	Deafness
Hematology	2° erythrocytosis		Anemia
Biochemistry	Mild or compensated hyperchloraemic acidosis	Severe hyperchloraemic acidosis	Hyperchloraemic acidosis
	Low/normal K	Low K	Low K
Urine chemistry	Min urine pH > 5.5	Min urine pH > 5.5	Min urine pH < 5.5[a]
	Hypercalciuria	Hypercalciuria	Bicarbonaturia
	Low urine citrate	Low urine citrate	
	Low urine ammonium	Low urine ammonium	
	Low urine-blood pCO_2	Low urine-blood pCO_2	High urine-blood pCO_2
Treatment	Citrate/bicarbonate	Citrate/bicarbonate	High dose citrate/bicarbonate
			Marrow/stem cell transplantation
Gene(s) and protein[b]	*SLC4A1*: AE1	*ATP6V1B1*: H^+-ATPase B1 subunit	*CA2*: Carbonic anhydrase II
		ATP6V0A4: H^+-ATPase a4 subunit	
		rarely *SLC4A1*: AE1	

[a] After bicarbonate replacement.
[b] Gene symbols for the *ATP6* family have been changed (www.gene.ucl.ac.uk/nomenclature/). *ATP6V1B1* replaces *ATP6B1*; *ATP6V0A4* replaces *ATP6N1B*.

interacts with cytoskeletal proteins such as 4.2, ankyrin, and spectrin (28).

Strikingly, a single base change alters the identical AE1 residue, R589, in eight of the ten reported kindreds with dominant dRTA, supporting the importance of this residue in the normal acidification process. R589 lies at the intracellular border of the sixth transmembrane domain of the protein, adjacent to K590. These basic residues are conserved in all the known vertebrate anion exchanger isoforms and are thought to form part of the site of intracellular anion binding. Another missense mutation alters serine to phenylalanine at position 613 (22) within the adjacent transmembrane loop, so this region of the protein is clearly important. A further complex mutation results in a C-terminally truncated AE1 protein lacking the last 11 amino acids (24,29).

It is notable that expression of these mutations in *Xenopus* oocytes did not generally result in sufficient loss of function to make haploinsufficiency the explanation for dRTA phenotype. Furthermore, mutations in *SLC4A1* cause the dominant red cell morphologic diseases hereditary spherocytosis and ovalocytosis, including mutations that result in very early termination or frameshift. These are not however usually associated with classical dRTA (30) (and dRTA is not associated with red cell fragility).

It has been suggested that the apparent dominant negative effects of *SLC4A1* mutations could be due to mistargeting of the mutant protein away from its normal basolateral location (31), but there are few data available concerning the motifs or binding partners that might direct targeting. Examination of kAE1's sequence yields some clues. One feature that merits attention is a YDEV motif in AE1's C-terminal tail, which is of the YXXØ type associated with adaptor protein interactions in polarized cells (32). Two recent reports have suggested that two of the reported mutant kAE1s (R589H and R901X) are retarded intracellularly when heterologously expressed in epithelial cells. These cells were not however polarized; they may not therefore represent the true *in vivo* steady state situation (29,33). The second of these mutations truncates the YXXØ region of the C-terminus, leaving intact the proximally adjacent CAII binding site (34). We have assessed this mutant protein by expression both in fully polarized MDCK and IMCD cells and found that in mature cells it is in fact targeted in a nonpolarized fashion to the cell surface (Devonald *et al.*, unpublished data).

This finding does not preclude there being additional targeting information in the R589 region of AE1 or motifs governing the interaction of AE1 with other intracellular components. For ex-

ample, in addition to CAII, kanadaptin has been shown to interact with AE1 *in vitro* (35), but to date there are few data of this sort.

AE1 in Autosomal Recessive dRTA

A few recessive kindreds from Thailand with coexistent hemolytic anemia and dRTA have now been described, with loss-of-function changes in AE1 that manifest in *in vitro Xenopus* but not red cell anion transport (36–38). This finding has resulted in some additional studies investigating the behavior of mutant AE1, because some mutations cause loss of function and others appear rather to result in intracellular retardation. However, whereas the red cell protein glycophorin A, which is not expressed in the kidney, can rescue mutant AE1 trafficking *in vitro* and may be an important partner in facilitating translocation of AE1 to the red cell surface (36), its mimic in the kidney remains to be identified. To date, the phenotypic combination of ovalocytosis and dRTA has not been reported in white patients and seems to be confined to Southeast Asia.

The Apical Proton Pump and Recessive dRTA

In contrast, genome-wide linkage analyses in a cohort of mainly consanguineous kindreds, largely of Middle Eastern and Turkish extraction, have localized two genes for recessive dRTA, on 2p and 7q (39,40).

The responsible genes have both been identified, accounting for types 1b (deaf) and 1c (hearing) disease respectively (39,4). Both encode kidney-specific subunits (in terms of major organs) of the high-density apical proton pump of α-IC: the B1 and a4 subunits, respectively (Figure 1).

DRTA with Deafness (Type 1b; MIM #267300)

ATP6V1B1, the gene encoding the B1-subunit of H^+-ATPase, resides on chromosome 2 and made an excellent candidate gene for dRTA because of its kidney specificity. Screening for mutations in this gene revealed fifteen different mutations in kindreds where almost all the affected individuals had documented bilateral SNHL and in all but one kindred were homozygous (39). The majority of these mutations are likely to disrupt the structure, or abrogate the production, of the normal B1 subunit protein. We also demonstrated expression of *ATP6V1B1* in the human cochlea and in mouse found it at the apical surface of interdental cells and in endolymphatic sac epithelium. Endolymph is a unique extracellular fluid, in that it has low Na^+ and high K^+ concentrations (41), which maximize the sensitivity of hair cells. To preserve its pH at 7.4, there is presumably a requirement for proton pumping into endolymph. It is thus assumed that H^+-ATPase containing the B1 subunit must contribute to this and that defects in B1 eventually cause irreversible hair cell damage because of ambient electrolyte and pH abnormalities. *ATP6V1B1* expression has also been observed in the male genital tract, another site with a particular acidification requirement for sperm maturation (42).

The recent creation of an *Atp6b1* null mouse has unfortunately not helped to evaluate the contribution of the B1 subunit in the ear or genital tract as yet, as these animals have normal hearing and fertility. Although not being spontaneously aci-

dotic (perhaps partly because of their alkaline diet), homozygotes do display a urinary acidification defect when acid-challenged (43).

It is, however, clear that in humans, the B2 subunit cannot substitute for B1 in either the IC surface H^+-ATPase or in inner ear pumps. In each H^+-ATPase molecule, three B subunits (either B1 or B2, but not both) associate with three A subunits and several other subunits, to form the catalytic head of the pump that hydrolyses ATP. The B subunit is necessary but not sufficient for this process (44). The B1 subunit notably contains a C-terminal membrane targeting motif (DTAL) that may be capable of interaction with PDZ domain proteins (45), and is absent from the ubiquitously expressed and usually intracellular B2 isoform. It is tempting to speculate that this region guides the pump to its final destination. However, the osteoclast surface pump does not contain B1, but this is a highly unusual cell type in which the ruffled border across which acid is secreted is not in fact the apical surface, and it remains unclear how the pump is guided at either location.

DRTA with Preserved Hearing (Type 1c; MIM #602722)

By a similar linkage approach in a cohort of dRTA kindreds in which hearing was essentially normal, the defective gene in this subset of families was found on chromosome 7 (*ATP6V0A4*). It encodes a newly identified kidney-specific a4 isoform of the proton pump's 116-kD accessory a subunit (4). Previously there had been disagreement as to whether renal proton pumps even contained an a subunit (46,47). Although the involvement of the a4 subunit in dRTA shows that it must be essential for proper proton pump function in the kidney, its role within the multi-subunit pump structure is at present unclear. Site-directed mutagenesis studies of the yeast a subunit ortholog Vph1p have yielded some potential functions. Some mutations showed that this subunit is important for the assembly of the proton pump, whereas other mutations had greater effects on ATPase activity and proton transport (48–50). These studies suggest that the a subunit is important for both assembly and function of the pump. Indeed by creating chimerae between Vph1p (the a subunit in proton pumps localized to the yeast vacuole) and Stv1p (the a subunit found in the proton pumps of yeast organelles), Kawasaki-Nishi *et al.* demonstrated that in yeast the amino terminal domain of the a subunit is important for targeting, whereas the carboxyl terminus seems to control the coupling of ATP hydrolysis to proton translocation (51). Further studies of the mammalian orthologs may be difficult to achieve *in vitro*, thanks to the complexity of the assembled pump.

Apart from the presence or absence of hearing loss, there do not appear to be major phenotypic differences at diagnosis between recessive patients with *ATP6V1B1* and *ATP6V0A4* mutations. With longer-term follow-up in our cohort of affected individuals, it has however become notably evident that mild and/or older-onset hearing impairment is present in some but as yet not all of those with *ATP6V0A4* mutations who were at first deemed to have normal hearing by audiometry. Consistent with this, we have recently determined that *ATP6V0A4*

is expressed in both adult and fetal human inner ear (Stover *et al.*, unpublished data).

Treatment of dRTA

Simple alkali replacement (by administration of 1 to 3 mmol/kg per d of citrate or bicarbonate orally) is usually sufficient to reverse most of the biochemical abnormalities and associated bone disease in both dominant and recessive dRTA, leading to the resumption of normal growth. Although additional potassium supplementation is not often required, K^+ salts are preferable to sodium, as the latter can exacerbate hypokalaemia. However, whereas alkali administration prevents further calcium deposition, it does not appear to either ameliorate or prevent the progression of the hearing impairment (52), presumably because of the anatomic isolation of the inner ear compartment.

Inherited Mixed RTA (Type 3; MIM #259730)

RTA with the characteristics of both proximal and distal tubular dysfunction usually accompanies one form of autosomal recessive osteopetrosis (Guibaud-Vainsel syndrome or marble brain disease). Here, both defective urinary acidification and bicarbonate wasting are usually observed, though the former may not become evident until alkali has been replaced. This condition is characterized by fractures, short stature, mental retardation, dental malocclusion, and visual impairment from optic nerve compression. Basal ganglion calcification may develop. Sly *et al.* identified loss of carbonic anhydrase II (CAII) as the biochemical defect (53), and CAII is known to be expressed not only by osteoclasts but in both proximal and distal nephron segments, explaining the mixed acidosis. Loss-of-function mutations have subsequently been described (54). The most common of these involves loss of the splice donor site in intron 2 of *CA2*, nicknamed the Arabic mutation (55). The presence of mental retardation and relative infrequency of skeletal fractures is said to distinguish the clinical course of patients with the Arabic mutation from that of patients with other *CA2* defects. It is also thought that the bone thinning effect of the coexistent metabolic acidosis in CAII deficiency goes some way to protecting the osteopetrotic bones from the excessive thickening generated by osteoclast dysfunction (56). Other than alkali supplementation, specific treatment requires bone marrow transplantation (57). This can reverse the bone phenotype, but has no effect on the RTA.

H^+K^+-ATPase and dRTA

As mentioned, the overall contribution of H^+K^+-ATPase to human renal function are unknown. Data from rabbits suggest multiple subunit isoforms (58), but neither the subunit isoform(s) present, nor their combination into functioning pumps in the human kidney, have been fully elucidated as yet (59). H^+K^+-ATPase are inhibitable by vanadate, and its administration to rats causes dRTA (60). Vanadate is present in high concentration in the soil in parts of the Far East, which may go some way to explaining the prevalence of dRTA there (61). A single infant was recently reported who presented with severe hypokalemic dRTA together with hypomagnesemia and nor-

mal gastric pH (62). The authors postulated that an H^+K^+-ATPase defect could explain the phenotype, but as yet, no human renal disorders of this type have been molecularly characterized.

Further Heterogeneity

Some families with primary recessive dRTA do not link to either *ATP6V1B1* or *ATP6V0A4*. There are numerous other candidate genes for recessive dRTA. These include (*1*) genes for all the known subunits of the proton transporters; (*2*) as yet unrevealed novel isoforms of these genes, particularly if they are kidney-specific; (*3*) genes with products that are required for trafficking of proton pumps to the apical membrane of the α-IC; and (*4*) genes encoding molecules necessary for the generation of protons, absorption of bicarbonate, recycling of chloride, or maintenance of the electrochemical gradient across both apical and basolateral membranes. Many, but by no means all, of these potential candidates have been identified, and developments in the human genome sequencing will undoubtedly facilitate future endeavors. The genetic identification of these natural human disease models has much to teach us about normal physiology and should lead to development of studies in related disciplines.

References

1. Forgac M: Structure, mechanism and regulation of the clathrin-coated vesicle and yeast vacuolar H(+)-ATPases. *J Exp Biol* 203: 71–80, 2000
2. Lu M, Holliday LS, Zhang L, Dunn WA Jr., Gluck SL: Interaction between aldolase and vacuolar H$^+$-ATPase: Evidence for direct coupling of glycolysis to the ATP-hydrolyzing proton pump. *J Biol Chem* 276: 30407–30413, 2001
3. Nelson RD, Guo XL, Masood K, Brown D, Kalkbrenner M, Gluck S: Selectively amplified expression of an isoform of the vacuolar H(+)- ATPase 56-kilodalton subunit in renal intercalated cells. *Proc Natl Acad Sci USA* 89: 3541–3545, 1992
4. Smith AN, Skaug J, Choate KA, Nayir A, Bakkaloglu A, Ozen S, Hulton SA, Sanjad SA, Al-Sabban EA, Lifton RP, Scherer SW, Karet FE: Mutations in *ATP6N1B*, encoding a new kidney vacuolar proton pump 116-kD subunit, cause recessive distal renal tubular acidosis with preserved hearing. *Nat Genet* 26: 71–75, 2000
5. Bowman EJ, Siebers A, Altendorf K: Bafilomycins: A class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. *Proc Natl Acad Sci USA* 85: 7972–7976, 1988
6. Schwartz GJ, Barasch J, Al-Awqati Q: Plasticity of functional epithelial polarity. *Nature* 318: 368–371, 1985
7. van Adelsberg J, Edwards JC, Takito J, Kiss B, al-Awqati Q: An induced extracellular matrix protein reverses the polarity of band 3 in intercalated epithelial cells. *Cell* 76: 1053–1061, 1994
8. Fejes-Toth G, Chen WR, Rusvai E, Moser T, Naray-Fejes-Toth A: Differential expression of AE1 in renal HCO$_3$-secreting and

-reabsorbing intercalated cells. *J Biol Chem* 269: 26717–26721, 1994

9. Royaux IE, Wall SM, Karniski LP, Everett LA, Suzuki K, Knepper MA, Green ED: Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. *Proc Natl Acad Sci USA* 98: 4221–4226, 2001

10. Parker MD, Ourmozdi EP, Tanner MJ: Human BTR1, a new bicarbonate transporter superfamily member and human AE4 from kidney. *Biochem Biophys Res Commun* 282: 1103–1109, 2001

11. Tsuganezawa H, Kobayashi K, Iyori M, Araki T, Koizumi A, Watanabe S, Kaneko A, Fukao T, Monkawa T, Yoshida T, Kim DK, Kanai Y, Endou H, Hayashi M, Saruta T: A new member of the $HCO_3(-)$ transporter superfamily is an apical anion exchanger of beta-intercalated cells in the kidney. *J Biol Chem* 276: 8180–8189, 2001

12. Wingo CS, Madsen KM, Smolka A, Tisher CC: H-K-ATPase immunoreactivity in cortical and outer medullary collecting duct. *Kidney Int* 38: 985–990, 1990

13. Lightwood R: Communication no. 1. *Arch Dis Child* 10: 205, 1935

14. Butler A, Wilson J, Farber S: Dehydration and acidosis with calcification at renal tubules. *J Pediatr* 8: 489, 1936

15. Batlle DC: Segmental characterization of defects in collecting tubule acidification. *Kidney Int* 30: 546–554, 1986

16. Walter SJ, Shirley DG, Unwin RJ, Wrong OM: Assessment of urinary acidification [Abstract]. *Kidney Int* 52: 2092, 1997

17. Agroyannis B, Koutsikos D, Tzanatos-Exarchou H, Yatzidis H: Erythrocytosis in type I renal tubular acidosis with nephrocalcinosis. *Nephrol Dial Transplant* 7: 365–366, 1992

18. Brown MT, Cunningham MJ, Ingelfinger JR, Becker AN: Progressive sensorineural hearing loss in association with distal renal tubular acidosis. *Arch Otolaryngol Head Neck Surg* 119: 458–460, 1993

19. Berrettini S, Neri E, Forli F, Panconi M, Massimetti M, Ravecca F, Sellari-Franceschini S, Bartolozzi C: Large vestibular aqueduct in distal renal tubular acidosis. High-resolution MR in three cases. *Acta Radiol* 42: 320–322, 2001

20. Phelps PD, Coffey RA, Trembath RC, Luxon LM, Grossman AB, Britton KE, Kendall-Taylor P, Graham JM, Cadge BC, Stephens SG, Pembrey ME, Reardon W: Radiological malformations of the ear in Pendred syndrome. *Clin Radiol* 53: 268–273, 1998

21. Chen A, Francis M, Ni L, Cremers CW, Kimberling WJ, Sato Y, Phelps PD, Bellman SC, Wagner MJ, Pembrey M, et al: Phenotypic manifestations of branchio-oto-renal syndrome. *Am J Med Genet* 58: 365–370, 1995

22. Bruce LJ, Cope DL, Jones GK, Schofield AE, Burley M, Povey S, Unwin RJ, Wrong O, Tanner MJ: Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene. *J Clin Invest* 100: 1693–1707, 1997

23. Jarolim P, Shayakul C, Prabakaran D, Jiang L, Stuart-Tilley A, Rubin HL, Simova S, Zavadil J, Herrin JT, Brouillette J, Somers MJ, Seemanova E, Brugnara C, Guay-Woodford LM, Alper SL: Autosomal dominant distal renal tubular acidosis is associated in three families with heterozygosity for the R589H mutation in the AE1 (band 3) Cl^-/HCO_3^- exchanger. *J Biol Chem* 273: 6380–6388, 1998

24. Karet FE, Gainza FJ, Gyory AZ, Unwin RJ, Wrong O, Tanner MJ, Nayir A, Alpay H, Santos F, Hulton SA, Bakkaloglu A, Ozen S, Cunningham MJ, di Pietro A, Walker WG, Lifton RP:

Mutations in the chloride-bicarbonate exchanger gene *AE1* cause autosomal dominant but not autosomal recessive distal renal tubular acidosis. *Proc Natl Acad Sci USA* 95: 6337–6342, 1998

25. Tanner MJ: The structure and function of band 3 (AE1): Recent developments [Review]. *Mol Membr Biol* 14: 155–165, 1997

26. Fujinaga J, Tang XB, Casey JR: Topology of the membrane domain of human erythrocyte anion exchange protein, AE1. *J Biol Chem* 274: 6626–6633, 1999

27. Lux SE, John KM, Kopito RR, Lodish HF: Cloning and characterization of band 3, the human erythrocyte anion-exchange protein (AE1). *Proc Natl Acad Sci USA* 86: 9089–9093, 1989

28. Tanner MJ: The major integral proteins of the human red cell. *Baillieres Clin Haematol* 6: 333–356, 1993

29. Toye AM, Bruce LJ, Unwin RJ, Wrong O, Tanner MJ: Band 3 Walton, a C-terminal deletion associated with distal renal tubular acidosis, is expressed in the red cell membrane but retained internally in kidney cells. *Blood* 99: 342–347, 2002

30. Jarolim P, Murray JL, Rubin HL, Taylor WM, Prchal JT, Ballas SK, Snyder LM, Chrobak L, Melrose WD, Brabec V, Palek J: Characterization of 13 novel band 3 gene defects in hereditary spherocytosis with band 3 deficiency. *Blood* 88: 4366–4374, 1996

31. Kaitwatcharachai C, Vasuvattakul S, Yenchitsomanus P, Thuwajit P, Malasit P, Chuawatana D, Mingkum S, Halperin ML, Wilairat P, Nimmannit S: Distal renal tubular acidosis and high urine carbon dioxide tension in a patient with southeast Asian ovalocytosis. *Am J Kidney Dis* 33: 1147–1152, 1999

32. Mostov KE, Verges M, Altschuler Y: Membrane traffic in polarized epithelial cells. *Curr Opin Cell Biol* 12: 483–490, 2000

33. Quilty JA, Li J, Reithmeier RA: Impaired trafficking of distal renal tubular acidosis mutants of the human kidney anion exchanger kAE1. *Am J Physiol Renal Physiol* 282: F810–820, 2002

34. Reithmeier RA: A membrane metabolon linking carbonic anhydrase with chloride/bicarbonate anion exchangers. *Blood Cells Mol Dis* 27: 85–89, 2001

35. Chen J, Vijayakumar S, Li X, Al-Awqati Q: Kanadaptin is a protein that interacts with the kidney but not the erythroid form of band 3. *J Biol Chem* 273: 1038–1043, 1998

36. Tanphaichitr VS, Sumboonnanonda A, Ideguchi H, Shayakul C, Brugnara C, Takao M, Veerakul G, Alper SL: Novel AE1 mutations in recessive distal renal tubular acidosis. Loss-of-function is rescued by glycophorin A. *J Clin Invest* 102: 2173–2179, 1998

37. Vasuvattakul S, Yenchitsomanus PT, Vachuanichsanong P, Thuwajit P, Kaitwatcharachai C, Laosombat V, Malasit P, Wilairat P, Nimmannit S: Autosomal recessive distal renal tubular acidosis associated with Southeast Asian ovalocytosis. *Kidney Int* 56: 1674–1682, 1999

38. Bruce LJ, Wrong O, Toye AM, Young MT, Ogle G, Ismail Z, Sinha AK, McMaster P, Hwaihwanje I, Nash GB, Hart S, Lavu E, Palmer R, Othman A, Unwin RJ, Tanner MJ: 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* 350: 41–51, 2000

39. Karet FE, Finberg KE, Nelson RD, Nayir A, Mocan H, Sanjad SA, Rodriguez-Soriano J, Santos F, Cremers CW, Di Pietro A, Hoffbrand BI, Winiarski J, Bakkaloglu A, Ozen S, Dusunsel R, Goodyer P, Hulton SA, Wu DK, Skvorak AB, Morton CC, Cunningham MJ, Jha V, Lifton RP: Mutations in the gene encoding the B1 subunit of H+-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet* 21: 84–90, 1999

40. Karet FE, Finberg KE, Nayir A, Bakkaloglu A, Ozen S, Hulton SA, Sanjad SA, Al-Sabban EA, Medina JF, Lifton RP: Localization of a gene for autosomal recessive distal renal tubular acidosis with normal hearing (*rdRTA2*) to 7q33–34. *Am J Hum Genet* 65: 1656–1665, 1999

41. Sterkers O, Saumon G, Tran Ba Huy P, Ferrary E, Amiel C: Electrochemical heterogeneity of the cochlear endolymph: Effect of acetazolamide. *Am J Physiol* 246: F47–53, 1984

42. Herak-Kramberger CM, Breton S, Brown D, Kraus O, Sabolic I: Distribution of the vacuolar H$^+$ ATPase along the rat and human male reproductive tract. *Biol Reprod* 64: 1699–1707, 2001

43. Finberg KE, Wang T, Wagner CA, Geibel JP, Dou H, Lifton RP: Generation and characterization of H$^+$ ATPase B1 subunit deficient mice [Abstract]. *J Am Soc Nephrol* 12: 3A–4A, 2001

44. Stone DK, Sun SZ, Xie XS: Structural properties of the proton translocating complex of the clathrin-coated vesicle. *Ciba Found Symp* 139: 238–251, 1988

45. Breton S, Wiederhold T, Marshansky V, Nsumu NN, Ramesh V, Brown D: The B1 subunit of the H$^+$ ATPase is a PDZ domain-binding protein. Colocalization with NHE-RF in renal B-intercalated cells. *J Biol Chem* 275: 18219–18224, 2000

46. Gluck S, Caldwell J: Immunoaffinity purification and characterization of vacuolar H$^+$ATPase from bovine kidney. *J Biol Chem* 262: 15780–15789, 1987

47. Gillespie J, Ozanne S, Tugal B, Percy J, Warren M, Haywood J, Apps D: The vacuolar H(+)-translocating ATPase of renal tubules contains a 115- kDa glycosylated subunit. *FEBS Lett* 282: 69–72, 1991

48. Leng XH, Manolson MF, Liu Q, Forgac M: Site-directed mutagenesis of the 100-kDa subunit (Vph1p) of the yeast vacuolar (H$^+$)-ATPase. *J Biol Chem* 271: 22487–22493, 1996

49. Leng XH, Manolson MF, Forgac M: Function of the COOH-terminal domain of Vph1p in activity and assembly of the yeast V-ATPase. *J Biol Chem* 273: 6717–6723, 1998

50. Kawasaki-Nishi S, Nishi T, Forgac M: Arg-735 of the 100-kDa subunit a of the yeast V-ATPase is essential for proton translocation. *Proc Natl Acad Sci USA* 98: 12397–12402, 2001

51. Kawasaki-Nishi S, Bowers K, Nishi T, Forgac M, Stevens TH: The amino-terminal domain of the vacuolar proton-translocating ATPase a subunit controls targeting and *in vivo* dissociation, and the carboxyl-terminal domain affects coupling of proton transport and ATP hydrolysis. *J Biol Chem* 276: 47411–47420, 2001

52. Zakzouk SM, Sobki SH, Mansour F, al Anazy FH: Hearing impairment in association with distal renal tubular acidosis among Saudi children. *J Laryngol Otol* 109: 930–934, 1995

53. Sly WS, Hewett-Emmett D, Whyte MP, Yu YS, Tashian RE: Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. *Proc Natl Acad Sci USA* 80: 2752–2756, 1983

54. Sly W, Shah GN: Osteopetrosis with renal tubular acidosis and cerebral calcification. In: *The Metabolic and Molecular Bases of Inherited Disease*, 8th edition, edited by Scriver, New York, McGraw-Hill, 2001, pp 5331–5343

55. Hu PY, Roth DE, Skaggs LA, Venta PJ, Tashian RE, Guibaud P, Sly WS: A splice junction mutation in intron 2 of the carbonic anhydrase II gene of osteopetrosis patients from Arabic countries. *Hum Mutat* 1: 288–292, 1992

56. Whyte MP, Murphy WA, Fallon MD, Sly WS, Teitelbaum SL, McAlister WH, Avioli LV: Osteopetrosis, renal tubular acidosis and basal ganglia calcification in three sisters. *Am J Med* 69: 64–74, 1980

57. McMahon C, Will A, Hu P, Shah GN, Sly WS, Smith OP: Bone marrow transplantation corrects osteopetrosis in the carbonic anhydrase II deficiency syndrome. *Blood* 97: 1947–1950, 2001

58. Caviston TL, Campbell WG, Wingo CS, Cain BD: Molecular identification of the renal H$^+$, K$^+$-ATPases. *Semin Nephrol* 19: 431–437, 1999

59. Kraut JA, Helander KG, Helander HF, Iroezi ND, Marcus EA, Sachs G: Detection and localization of H$^+$-K$^+$-ATPase isoforms in human kidney. *Am J Physiol Renal Physiol* 281: F763–F768, 2001

60. Dafnis E, Spohn M, Lonis B, Kurtzman NA, Sabatini S: Vanadate causes hypokalemic distal renal tubular acidosis. *Am J Physiol* 262: F449–F453, 1992

61. Sitprija V, Tungsanga K, Eiam-Ong S, Leelhaphunt N, Sriboonlue P: Renal tubular acidosis, vanadium and buffaloes. *Nephron* 54: 97–98, 1990

62. Simpson AM, Schwartz GJ: Distal renal tubular acidosis with severe hypokalaemia, probably caused by colonic H(+)-K(+)-ATPase deficiency. *Arch Dis Child* 84: 504–507, 2001

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