Distal Renal Tubular Acidosis in Mice Lacking the AE1 (Band3) Cl^-/HCO_3^- Exchanger (*slc4a1*)

Paul A. Stehberger,* Boris E. Shmukler,† Alan K. Stuart-Tilley,†
Luanne L. Peters,‡ Seth L. Alper,†§ and Carsten A. Wagner*

*Institute of Physiology and Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland; †Molecular and Vascular Medicine Unit and Renal Division, Beth Israel Deaconess Medical Center, and §Department of Medicine, Harvard Medical School, Boston, Massachusetts; and ‡Jackson Laboratories, Bar Harbor, Maine

Mutations in the human gene that encodes the AE1 Cl^-/HCO_3^- exchanger (*SLC4A1*) cause autosomal recessive and dominant forms of distal renal tubular acidosis (dRTA). A mouse model that lacks AE1/slc4a1 (*slc4a1*−/−) exhibited dRTA characterized by spontaneous hyperchloremic metabolic acidosis with low net acid excretion and, inappropriately, alkaline urine without bicarbonaturia. Basolateral Cl^-/HCO_3^- exchange activity in acid-secretory intercalated cells of isolated superfused *slc4a1*−/− medullary collecting duct was reduced, but alternate bicarbonate transport pathways were upregulated. Homozygous mice had nephrocalcinosis associated with hypercalciuria, hyperphosphaturia, and hypocitraturia. A severe urinary concentration defect in *slc4a1*−/− mice was accompanied by dysregulated expression and localization of the aquaporin-2 water channel. Mice that were heterozygous for the AE1-deficient allele had no apparent defect. Thus, the *slc4a1*−/− mouse is the first genetic model of complete dRTA and demonstrates that the AE1/*slc4a1* Cl^-/HCO_3^- exchanger is required for maintenance of normal acid-base homeostasis by distal renal regeneration of bicarbonate in the mouse as well as in humans.

J Am Soc Nephrol 18: 1408–1418, 2007. doi: 10.1681/ASN.2006101072

Normal kidney function is critical for maintenance and control of systemic acid-base homeostasis, as highlighted by several inborn and acquired diseases (1–5). Three main activities contribute to this role: Proximal tubular reabsorption of filtered bicarbonate, proximal tubular synthesis and excretion of ammonia, and collecting duct excretion of protons and regeneration of bicarbonate. Type A acid-secretory intercalated cells (A-IC) in the collecting duct express an apical vacuolar H^+-ATPase and a basolateral Cl^-/HCO_3^- exchanger kAE1 (kidney band 3, SLC4A1) that respectively mediate proton extrusion into urine and bicarbonate release into blood (2,6). Bicarbonate is formed in the A-IC by the cytosolic carbonic anhydrase. The concerted action of these transport proteins and enzymes in A-IC is important for acid secretion and for parallel *de novo* generation of bicarbonate and/or its reabsorption from the luminal fluid.

AE1 is expressed in red blood cells and in renal A-IC. The kidney-specific kAE1 gene product lacks the N-terminal 79 amino acids (aa) that are present in mouse erythroid AE1 (eAE1), as a result of alternative promoter usage (7,8). Human kAE1 lacks the N-terminal 65 aa that are present in human eAE1 (6). The N-terminal extension of eAE1 polypeptide is important for interaction with erythroid-specific cytoskeletal and membrane proteins. One group of human AE1 mutations causes dominantly transmitted erythroid-specific abnormalities such as hereditary spherocytic anemia, Southeast Asian ovalocytosis (SAO), and other "hereditary stomatocytoses with cation leak" (9) accompanied by normal renal function. A distinct set of AE1 mutations can cause distal renal tubular acidosis (dRTA) without apparent erythroid abnormality (1,10–14).

dRTA-associated AE1 mutations are predominantly autosomal dominant among white individuals (13,15,16), with disease characterized by hyperchloremic metabolic acidosis, variable hypokalemia, osteomalacia, childhood growth retardation, nephrocalcinosis, and nephrolithiasis progressing to renal insufficiency (5,15,17). At least two pathophysiologic mechanisms have been proposed to explain autosomal dominant dRTA as a result of AE1 mutations (1,9): A dominant negative intracellular retention phenotype (18) and a dominant or co-dominant mistargeting to the apical membrane (19,20). A distinct set of mutations found among Southeast Asians and Melanesians is transmitted in autosomal or compound recessive patterns (11,14). The recessive disease is often of earlier onset and accompanied by a urinary concentrating defect, with affected infants susceptible to severe dehydration (1,3,5,15,17,21–23). Rare recessive mutations cause combined early-onset hemolytic anemia and dRTA manifest as hydrops fetalis (24). The severity of these symptoms emphasizes the importance of AE1 for normal kidney function. In this study, we examined the renal phenotype of AE1-deficient mice to gain additional insight into the physiologic function of kAE1, the pathogenesis of dRTA

Received October 2, 2006. Accepted February 14, 2007.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. Carsten A. Wagner, Institute of Physiology and Zurich Center for Integrative Human Physiology, University of Zurich, Winterthurerstrasse 190, CH-807 Zurich, Switzerland. Phone: +41-44-63-50569; Fax: +41-44-63-56814; E-mail: wagnerca@access.unizh.ch

ISSN: 1046-6673/1805-1408

as a result of AE1 gene mutations, and the mode of inheritance of AE1 mutation–associated dRTA.

Materials and Methods

Animals

Generation and breeding of mice that were deficient for anion exchanger 1 (*Slc4a1* −/−) on a C57BL/6J background was previously described (25). Mice were maintained on a standard rodent chow (Kliba AG, Kaiseraugst, Switzerland) and had access to drinking water *ad libitum*.

Food and water consumption was monitored, and urine and stool output was quantified in metabolic cages, with urine collection under mineral oil. After an adaptation period to the metabolic cage environment, experiments were performed over periods of 1 to 3 d. During days 1 and 2, mice were kept under control conditions (tap water with 2% sucrose). On day 3, 0.28 M NH_4Cl was added to the drinking water as an oral acid challenge (26,27). All animal studies were according to Swiss Animal Welfare Laws and approved by the Local Veterinary Authority (Kantonales Veterinäramt) Zurich.

Blood and Urine Analysis

Blood and urine samples that were obtained from metabolic cage experiments were injected into a blood gas analyzer (ABL 505, Radiometer Copenhagen, Copenhagen, Denmark) for determination of pH, Pco_2, calculated [HCO_3^-], K^+, Na^+, Cl^- (blood), and HCO_3^- (urine). pH of urine that was collected under oil was measured using a pH microelectrode (Metrohm, Herisau, Switzerland). Urine was diluted in distilled water for anion determinations by ion chromatography (Metrohm ion chromatograph) on a SUPP 5-250 column eluted with 3.2 mM Na_2CO_3/1 mM $NaHCO_3$. Urine was diluted in 4 mM tartaric acid for determination of K^+, Na^+, Ca^{2+}, and Mg^{2+} on a METROSEP C-2-150 column eluted with 4 mM tartaric acid/1 mM dipicolinic acid. Serum creatinine concentration was measured using a kit based on the F-Daos method (Wako Chemicals, Neuss, Germany). Urine creatinine was analyzed by the Jaffé method (28,29). Urine ammonium concentration was determined according to the Berthelot Protocol (30). Urinary citrate concentration was measured with the kit from R-Biopharm (Murten, Switzerland). Titratable acids were determined according to Chan (31). Briefly, equal volumes of urine and 0.1 N HCl were mixed and boiled for 2 min. After 10 min of cooling to 37°C, the mix was titrated with 0.1 N NaOH to pH 7.4. A blank sample (distilled water) was identically treated. The difference in volumes of NaOH required to titrate the sample and the blank, multiplied by the normality of NaOH times 1000, revealed the concentration of titratable acids in mmol/L. Osmolarity of blood and urine was determined using a Roebling freezing point osmometer (Auer Bittmann Soulié, Dietikon, Switzerland). GFR, fractional excretion of ions, anion gap in blood and urine, and net acid excretion were calculated from the measured values.

Statistical Analyses

All data were tested for significance using the ANOVA and *t* test. Only data with $P < 0.05$ were considered as significantly different.

Results

The renal phenotype of AE1-deficient mice was not analyzed in early studies because of high perinatal and postnatal lethality (Supplementary Figure 2) (25,32). Crossing of the few surviving *Ae1*−/− male homozygote mice with *Ae1*+/− females resulted in AE1-deficient *Ae1*−/− progeny that exhibited approximately 16% postweaning survival to the age of 10 to 14 wk. AE1-deficient neonates were smaller than their littermates and required 5 to 6 wk of age before they could be successfully weaned. Surviving AE1-deficient mice grew more slowly than heterozygous or wild-type animals, with *Ae1*−/− male weight at 12 wk of 22.6 ± 1.1 g ($n = 8$) *versus Ae1*+/+ weight of 26.3 ± 1.7 g ($n = 9$).

At age 5 to 7 d, the *Ae1*−/− mouse hematocrit measured on two separate occasions was 7.1 ± 1.0% ($n = 9$) and 13.3 ± 1.0% ($n = 18$), with reticulocytosis of >70%. In six additional mice of mean age 14 wk, hematocrit was 10.9 ± 0.5%. These severely anemic values are nonetheless slightly higher than those originally reported (25).

Twelve-week-old mice were used to assess consequences of the congenital absence of AE1 on renal acid-base transport and systemic acid-base homeostasis. Immunoblotting with an antibody directed against an epitope that was common to the erythrocyte and kidney isoforms confirmed the complete absence of AE1 from kidneys of AE1-deficient mice (Supplementary Figure 1). Kidney function and systemic acid-base balance were evaluated under basal conditions and during a 24-h oral acid load. Under control conditions (Table 1, Figure 1) wild-type and *slc4a1*+/− mice exhibited no apparent difference in blood or urine parameters. In contrast, *slc4a1*−/− mice had severe metabolic acidosis with low blood pH and bicarbonate levels and slightly increased blood chloride and phosphate levels (Table 1). Elevated serum urea levels pointed to mild renal insufficiency in AE1-deficient mice, and elevated serum osmolarity suggested mild systemic dehydration. The urine of AE1-deficient mice was alkaline, with reduced excretion of net acid and of titratable acids (Table 1, Figure 1). Twenty-four-hour urine volume of *Ae1*−/− mice was two- to three-fold higher than for wild-type and heterozygous mice, with correspondingly lower osmolality. In *Ae1*−/− mice, hypercalciuria and hypocitraturia were also evident in basal conditions.

Challenge with an oral acid load (NH_4Cl added to drinking water) induced metabolic acidosis in wild-type and heterozygous mice, as evident from reduced blood pH and bicarbonate levels, hyperchloremia, and decreased urine pH with increased net acid excretion (Table 2, Figure 1). In AE1-deficient mice, this NH_4Cl-loading protocol disproportionately exacerbated the basal metabolic acidosis, decreasing blood pH to 6.82 ± 0.02 (compared with 7.21 ± 0.01 in wild-type and 7.20 ± 0.01 in heterozygous mice; $n = 7$ to 9). The severely decreased blood bicarbonate concentration in AE1-deficient mice was accompanied by a less than proportionate reduction in Pco_2, consistent with a partial respiratory compensation (Tables 1 and 2, Figure 1). Despite the severe metabolic acidosis in *slc4a1*−/− mice, urine pH remained more alkaline and net acid excretion remained much lower than in wild-type littermates.

NH_4Cl loading had to be terminated after 24 h. Longer

Table 1. Control conditions[a]

Genotype	*Ae1*+/+	*Ae1*+/−	*Ae1*−/−
Blood			
pH	7.27 ± 0.02	7.30 ± 0.02	7.12 ± 0.02[b]
HCO_3^-	22.0 ± 0.8	22.2 ± 0.3	17.3 ± 0.5[b]
Pco_2	48.8 ± 1.7	44.9 ± 1.0	55.8 ± 2.1
K^+	3.7 ± 0.1	3.5 ± 0.0	4.4 ± 0.1[c]
Na^+	147.3 ± 1.1	145.2 ± 0.8	149.1 ± 1.5
Cl^-	113.3 ± 1.1	112.2 ± 1.1	116.7 ± 1.2[c]
P_i	1.6 ± 0.1	1.6 ± 0.1	2.4 ± 0.3[c]
anion gap	12.0 ± 0.8	10.8 ± 0.2	15.1 ± 1.1
osmolarity	315.1 ± 2.6	310.2 ± 1.5	336.0 ± 9.1[c]
creatinine	0.07 ± 0.01	ND	0.11 ± 0.02
urea	6.4 ± 1.6	ND	26.8 ± 3.8[b]
Urine			
pH	6.48 ± 0.16	6.38 ± 0.13	6.77 ± 0.12
osmolarity	2519.8 ± 176.3	2336 ± 279.1	1085.1 ± 103.1[b]
creatinine	68.9 ± 3.8	66.2 ± 6.6	27.1 ± 3.4[b]
HCO_3^-/creatinine	0.2 ± 0.1	0.1 ± 0.0	0.5 ± 0.2
K^+/creatinine	7.4 ± 1.0	2.9 ± 0.4[c]	6.9 ± 1.1
Na^+/creatinine	3.5 ± 0.5	1.2 ± 0.2[c]	3.3 ± 0.5
Cl^-/creatinine	3.8 ± 0.3	3.7 ± 0.6	4.2 ± 0.3
P_i/creatinine	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
NH_4^+/creatinine	1.2 ± 0.2	1.0 ± 0.2	1.4 ± 0.2
Ca^{2+}/creatinine	0.05 ± 0.01	0.05 ± 0.01	0.11 ± 0.02[c]
Mg^{2+}/creatinine	0.44 ± 0.09	0.19 ± 0.04	0.66 ± 0.19
citrate/creatinine	0.71 ± 0.09	ND	0.25 ± 0.04[b]
creatinine clearance	1.03 ± 0.17	ND	1.11 ± 0.22
FE Na^+ (%)	0.26 ± 0.05	ND	0.68 ± 0.15[c]
FE K^+ (%)	21.25 ± 4.11	ND	10.31 ± 2.23
FE Cl^- (%)	0.31 ± 0.08	ND	0.31 ± 0.05
FE Ca^{2+} (%)	0.76 ± 0.09	ND	0.58 ± 0.08
FE Mg^{2+} (%)	5.62 ± 2.76	ND	6.88 ± 1.16
FE P_i (%)	2.09 ± 0.42	ND	1.81 ± 0.44
urinary anion gap	177.8 ± 89.3	21.4 ± 36.3	36.8 ± 24.2
titratable acids	38.7 ± 4.6	28.0 ± 6.1	5.5 ± 6.6[b]
NEA	120.0 ± 10.2	94.1 ± 14.6	49.1 ± 12.4[b]
NEA/creatinine	1.9 ± 0.2	1.4 ± 0.1	1.6 ± 0.4

[a]Summary of basal blood and urine data. Urine was collected during 24 h in metabolic cages, and blood was sampled at the end of the collection period. Concentrations of electrolytes, titratable acids, and urea are expressed in units of mmol/L, creatinine in mg/dl, Pco_2 in mmHg, osmolarity in mOsmol/L, creatinine clearance in ml/min; n = 8 to 10 animals per genotype. ND, not determined; NEA, net acid excretion.

[b]$P < 0.001$ *versus* wild-type.

[c]$P < 0.05$ *versus* wild-type.

periods of acid loading led to death of AE1-deficient mice from apparent dehydration (weight loss) and massive hemolysis (dark urine as a result of hemoglobulinuria; Supplementary Figure 3). The severe dehydration in acid-loaded AE1-deficient mice was reflected in elevated serum osmolarity and elevated serum concentrations of sodium and chloride. It is interesting that heterozygous mice failed to display a wild-type urinary concentration response to the oral acid load.

The acid-base parameters of wild-type and heterozygous mice did not differ in their responses to acid loading. Whereas haploinsufficiency of AE1 did not affect basal systemic acid-base status, complete absence of AE1 led to hyperchloremic metabolic acidosis and to lethal intolerance of an oral acid load easily borne by heterozygotes.

Intercalated cells (IC) were maintained in the collecting ducts of 9-wk-old *Ae1*−/− mice. The proportion of cortical collecting duct (CCD) IC that were type A-IC (measured as pendrin-negative, H^+-ATPase–positive cells) was 48% in 516 A-IC cells from two *Ae1*−/− kidneys and 48% in 715 A-IC from one wild-type kidney. The proportion of A-IC was also indistin-

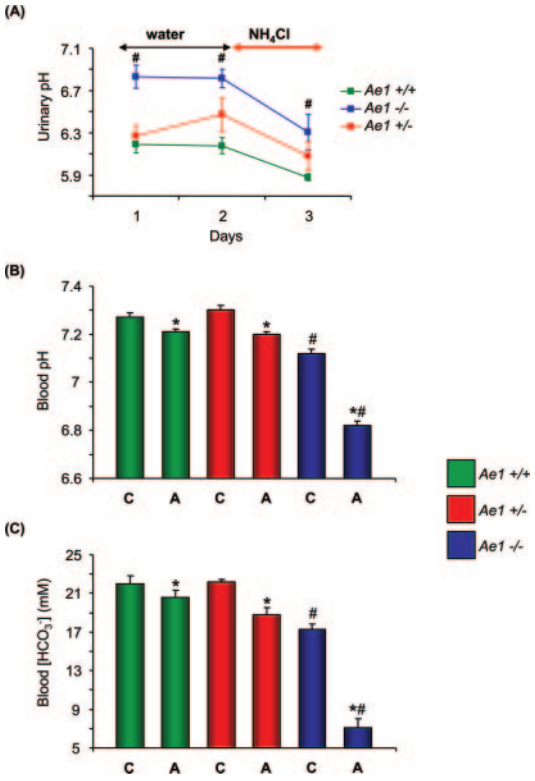

Figure 1. Urine and blood gas analysis. Urinary pH and blood gas values of wild-type (*Ae1*+/+), heterozygous (*Ae1*+/−) and AE1-deficient (*Ae1*−/−) mice during metabolic cage experiments under control conditions for 2 d and after oral acid loading with 0.28 M NH_4Cl for a third day. (A) Urinary pH in AE1-deficient mice was more alkaline than for other genotypes in all conditions but did acidify in response to the oral NH_4Cl load. Blood pH (B) and [HCO_3^-] concentration (C) in wild-type and heterozygous mice were indistinguishable in all conditions. However, blood pH and [HCO_3^-] in *Ae1*−/− mice under control conditions revealed metabolic acidosis. This acidosis was severely exacerbated after NH_4Cl loading ($n = 8$ mice for each genotype and time point). $^*P < 0.001$ *versus* control conditions in the same genotype; $^{\#}P < 0.001$ *versus* wild-type.

guishable when assessed by apical *versus* diffuse localization of H^+-ATPase (data not shown) and in kidneys of 5-wk-old mice.

We directly compared Cl^-/HCO_3^- exchanger activity in type A-IC of freshly isolated outer medullary collecting ducts from wild-type and AE1-deficient mice. Activity was measured in 2′-7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescin, acetoxymethyl ester (BCECF)-loaded tubules as the initial rate of intracellular acidification upon chloride readdition to chloride-free bath in the presence of 5% CO_2/25 mM HCO_3^- (33). The substantial acidification that was observed in wild-type A-IC was inhibited approximately 60% by 400 nM diBA(5)C4 (34) and by 50 μM 4-4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), consistent with basolateral membrane AE1-mediated Cl^-/HCO_3^- exchange activity (35–37). In contrast, A-IC from AE1-deficient mice exhibited a modestly decreased acidification rate of approximately 80% the wild-type value A-IC (wild-type −0.162 ± 0.011 units pH/min [$n = 164$ A-IC in six outer medullary collecting ducts (OMCD) from 4 mice *versus* AE1 −/− −0.123 ± 0.005 units pH/min [$n = 246$ type A-IC in seven OMCD from four mice; Figure 2). However, acidification by *slc4a1*−/− A-IC was insensitive to diBA(5)C4 and showed reduced DIDS sensitivity (inhibition by approximately 20%), suggesting upregulation of a pharmacologically distinct anion exchange activity.

Among candidate anion exchangers that might contribute to this apparent compensation are Ae2/Slc4a2 (38–40), Ae3/slc4a3, Ae4/Slc4a9 (41), SLC26A7 (37), and (if atypically induced in A-IC) pendrin/Slc26a4 (42–45). Renal levels of AE2 and AE3 transcript variants (Figure 3A) as well as AE4 mRNA abundance (data not shown) were not elevated in *slc4a1*−/− mice (Figure 3A). Unchanged renal mRNA levels of AE2 variant transcripts were confirmed by RNase protection assay (data not shown). AE4 polypeptide levels were also unchanged in *slc4a1*−/− kidney, consistent with previously reported lack of sensitivity to acid loading in wild-type mice (41) (Figure 3B). However, levels of pendrin polypeptide were markedly decreased, consistent with the previously reported sensitivity of wild-type mouse kidney pendrin to acid loading (42,44,46). In addition, no consistent changes were found in *slc4a1*−/− kidney levels of mRNA encoding A-IC gene products Slc26A7 (37), aquaporin-6 (AQP6) (47), the K^+/Cl^- cotransporter KCC4 (48), and the renal anion exchanger Slc26A11 (49), whose nephron segment localization is unreported.

Basal 24-h urine volume in AE1-deficient mice was three- to four-fold higher than the wild-type and heterozygote values. Urine osmolality was correspondingly approximately 50% lower than in wild-type and heterozygous mice (Tables 1 and 2, Figure 4). The accompanying elevation in plasma osmolarity in *slc4a1*−/− mice suggested a urinary concentrating defect. NH_4Cl loading led to loss of 14% of initial body weight within 24 h and to greatly increased plasma osmolarity without the accompanying increase in urine osmolality seen in wild-type mice (Figure 4). Basal creatinine clearance was similar in all genotypes but decreased greatly in AE1-deficient mice during acidosis (Tables 1 and 2), likely reflecting systemic volume depletion and dehydration.

We therefore examined expression and localization of AQP2 under control conditions. In AE1-null mice, AQP2 abundance was increased slightly in cortex and substantially in medulla, consistent with the observed systemic dehydration. Most inner medullary AQP2 was confined to cytoplasmic vesicular structures, in contrast to the predominantly luminal membrane localization in wild-type mice. In contrast, the less dramatically increased AQP2 of OMCD was normally localized to the luminal membrane in both *slc4a1*−/− and *slc4a1*+/+ mice (Figure 5). Gross kidney morphology revealed reduced inner medullary mass, resembling that of chronic hydronephrosis. Thus, the urinary concentrating defect of AE1-null mice is associated with defective inner medullary AQP2 trafficking in the setting of inner medullary atrophy, with a compensatory increase in abundance of outer medullary AQP2.

Distal renal tubular acidosis is often associated with nephrocalcinosis and nephrolithiasis (3,15,23,50). The elevated fractional excretion of calcium in AE1-null mice is (as in humans) a risk factor for nephrocalcinosis (Tables 1 and 2, Figure 6). Urinary citrate is thought to protect against nephrocalcinosis,

Table 2. 24-h NH_4Cl loading[a]

Genotype	*Ae1*+/+	*Ae1*+/−	*Ae1*−/−
Blood			
pH	7.21 ± 0.01	7.20 ± 0.01	6.82 ± 0.05[b]
HCO_3^-	20.6 ± 0.7	18.8 ± 0.7	7.1 ± 0.9[b]
Pco_2	53.3 ± 1.8	49.6 ± 1.5	44.4 ± 3.1[c]
K^+	3.7 ± 0.2	4.0 ± 0.1	4.9 ± 0.3[b]
Na^+	147.9 ± 1.0	148.1 ± 1.0	156.4 ± 2.8[c]
Cl^-	117.0 ± 1.8	119.9 ± 2.0	133.0 ± 4.3[b]
P_i	2.0 ± 0.2	2.1 ± 0.2	3.4 ± 0.1[b]
anion gap	10.1 ± 1.8	9.5 ± 2.3	16.3 ± 2.4[c]
osmolarity	304.1 ± 1.2	302.4 ± 1.5	346.8 ± 3.4[b]
creatinine	0.08 ± 0.02	0.10 ± 0.02	0.15 ± 0.02[c]
urea	10.8 ± 1.6	ND	29.6 ± 5.3[b]
Urine			
pH	6.07 ± 0.06	6.10 ± 0.10	6.47 ± 0.12[c]
osmolarity	3302.5 ± 178.4	2388 ± 97.1	1176.5 ± 69.7[b]
creatinine	83.1 ± 5.8	86.1 ± 13.1	33.2 ± 3.3[b]
HCO_3^-/creatinine	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
K^+/creatinine	3.5 ± 0.6	3.1 ± 0.7	5.2 ± 1.0
Na^+/creatinine	1.8 ± 0.4	1.4 ± 0.3	3.5 ± 0.7
Cl^-/creatinine	7.6 ± 0.5	7.3 ± 1.0	6.7 ± 1.0
P_i/creatinine	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
NH_4^+/creatinine	2.1 ± 0.2	2.2 ± 0.3	2.2 ± 0.3
Ca^{2+}/creatinine	0.04 ± 0.01	0.05 ± 0.02	0.06 ± 0.01
Mg^{2+}/creatinine	0.19 ± 0.04	0.20 ± 0.08	0.26 ± 0.07
creatinine clearance	1.46 ± 0.40	0.80 ± 0.31	0.47 ± 0.06[b]
FE Na^+ (%)	0.10 ± 0.03	0.09 ± 0.05	0.34 ± 0.08[c]
FE K^+ (%)	6.78 ± 2.06	3.99 ± 1.05	16.72 ± 4.06
FE Cl^- (%)	0.44 ± 0.10	0.58 ± 0.22	0.76 ± 0.15
FE Ca^{2+} (%)	0.15 ± 0.06	0.24 ± 0.11	0.31 ± 0.07
FE Mg^{2+} (%)	4.42 ± 1.69	2.41 ± 1.78	3.80 ± 1.08
FE P_i (%)	1.98 ± 0.43	3.02 ± 1.01	3.52 ± 0.62
urinary anion gap	−202.3 ± 69.7	−223.2 ± 57.6	80.6 ± 61.8[c]
titratable acids	37.8 ± 3.2	20.0 ± 4.7	16.1 ± 4.4[c]
NEA	205.7 ± 9.5	176.6 ± 10.2	85.2 ± 5.2[b]
NEA/Crea	2.6 ± 0.2	2.9 ± 0.5	2.7 ± 0.3

[a]Summary of blood and urine data after 24 h of oral NH_4Cl loading. Urine was collected during 24 h in metabolic cages, and blood was sampled at the end of the collection period. Concentrations of electrolytes, titratable acids, and urea are expressed in units of mmol/L, creatinine in mg/dl, Pco_2 in mmHg, osmolarity in mOsmol/L, creatinine clearance in ml/min; n = 8 to 10 animals per genotype.
[b]$P < 0.001$ *versus* wild-type.
[c]$P < 0.05$ *versus* wild-type.

and its reduced excretion in acidosis is attributed to upregulation of the NaDC1 Na^+/citrate co-transporter (51). The hypocitraturia of AE1-deficient mice (Table 1, Figure 6) constitutes an additional risk for nephrocalcinosis. Indeed, Van Kossa staining of *slc4a1*−/− kidneys demonstrated predominantly medullary calcium-phosphate deposits (Figure 6), a distribution typical of human dRTA. However, nephrolithiasis was not observed in *slc4a1*−/− mice.

Discussion

Mutations in the human *SLC4A1/AE1* gene cause familial dRTA (1,15,17). The attendant hyperchloremic metabolic acidosis often presents as childhood growth retardation with increased risk for dehydration, accompanied by hypokalemia and nephrocalcinosis (1,3,15,17). The AE1-deficient mouse model examined here reproduces many features of the human disease and therefore represents the first mouse model of an inborn form of complete dRTA. A mouse that lacks the IC-specific vacuolar H^+-ATPase B1 subunit (*Atp6v1b1*−/−) shows only incomplete dRTA, with oral acid loading required to unmask a modest urinary acidification defect (52). This mild phenotype contrasts with the severe and often early-onset complete dRTA of patients with mutations in the orthologous human *ATP6V1B1* gene (1,6,53).

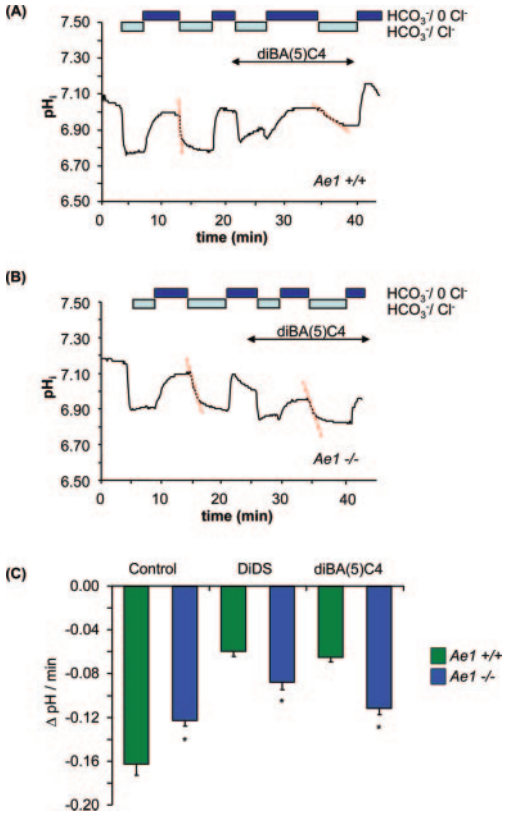

Figure 2. Measurement of Cl^-/HCO_3^- exchanger activity in single type A intercalated cells (A-IC) in isolated outer medullary collecting duct (OMCD). (A and B) Representative original tracings of intracellular pH measurements (BCECF ratio fluorimetry) in the presence of HCO_3^-/CO_2 and the alternating presence or absence of extracellular chloride. Addition of chloride caused a rapid acidification as a result of activation of Cl^-/HCO_3^- exchange activity. The intracellular acidification rate was reduced by 400 nM of the AE1 inhibitor diBA(5)C4. Intracellular acidification was partially reduced in A-IC from *Ae1−/−* mice, and the remaining activity was insensitive to diBA(5)C4. (C) Summarized data from all experiments using the inhibitors diBA(5)C4 (400 nM) or DIDS (50 μM; $n = 4$ to 5 mice with five to six OMCD for each condition and 100 to 150 A-IC each). *$P < 0.001$ *versus* wild-type.

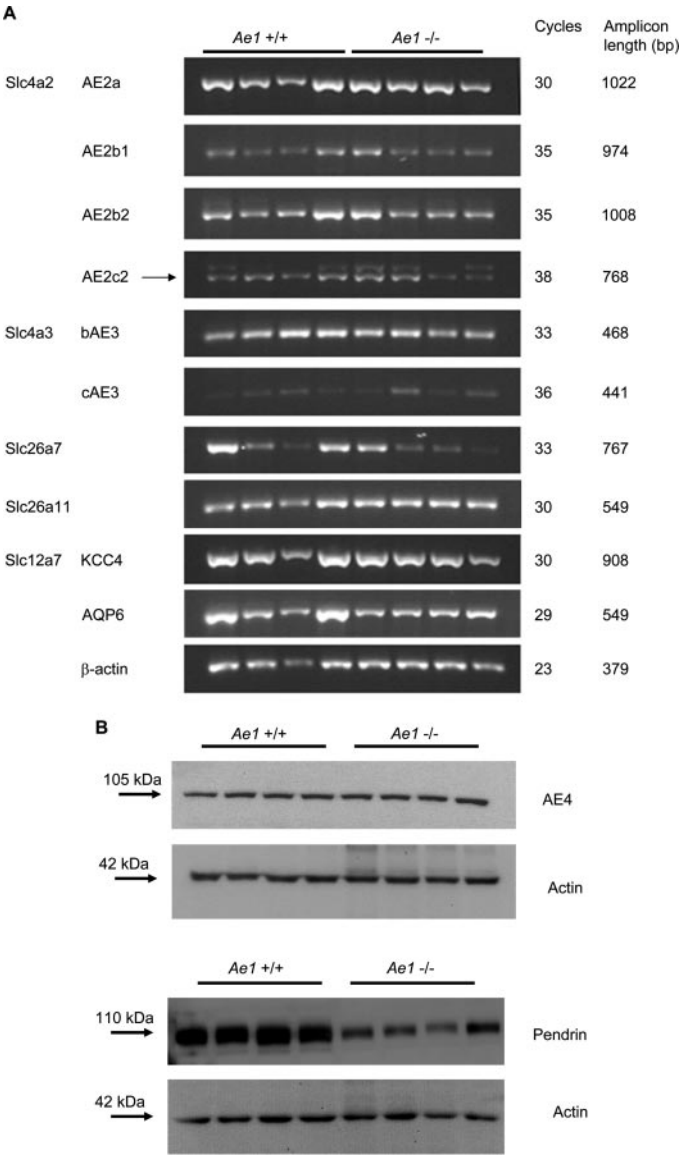

Figure 3. Expression of anion exchangers in kidney. (A) reverse transcriptase–PCR with primers detecting specific isoforms of several anion exchanger mRNA as well as KCC4 and aquaporin-6 (AQP6), two transport proteins implicated in A-IC function, did not reveal consistent changes in mRNA abundance in kidneys from *Ae1−/−* mice. β-Actin was used as a reference transcript. (B) Immunoblot showed that abundance of AE4/Slc4a9 polypeptide was not changed in kidneys from *Ae1−/−* mice. The abundance of pendrin/Slc26a4 anion exchanger polypeptide was strongly reduced in kidneys from *Ae1−/−* mice.

A-IC of the collecting duct are believed to complete the reabsorption of filtered bicarbonate in excess of the reabsorptive capacities of the proximal tubule and the thick ascending limb (2). The absence of bicarbonaturia in the face of the metabolic acidosis of AE1-deficient mice suggests (at least in the presence of reduced blood bicarbonate levels) that AE1 is not critical for reabsorption of filtered bicarbonate. Thus, the main function of A-IC may be rather to replenish body bicarbonate that is newly generated by cytosolic carbonic anhydrase and to release it into blood *via* AE1 and perhaps other basolateral anion exchangers.

Although of considerable magnitude, the residual activity of alternative chloride-bicarbonate exchange activity in A-IC of the *slc4a1−/−* mouse apparently does not suffice to support adequate bicarbonate release into the blood. The compensatory anion exchange activity was measured in unperfused OMCD tubules at isotonic bath of pH 7.4 and therefore may not optimally reflect conditions in the acidotic, moderately hypertonic outer medulla of the *AE1−/−* mouse. The molecular identity, regulation, and physiologic function of the alternate anion exchanger(s) remain to be elucidated. Potential candidate anion exchangers reported previously in A-IC basolateral membrane include AE4/Slc4a9 and Slc26a7. Although AE4 has been local-

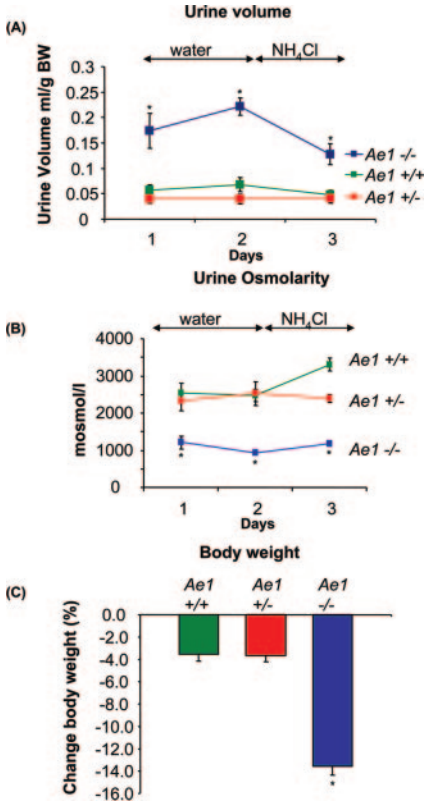

Figure 4. Severe urinary concentrating defect in *Ae1−/−* mice. (A) Under control conditions, urine volume normalized to body weight was approximately three-fold elevated in AE1-deficient mice and remained two-fold elevated during the severe dehydration produced by acid loading (see serum osmolarity, Table 2). (B) Urine osmolarity was much lower in AE1-deficient mice and did not increase during acid load–induced dehydration. (C) The acid load–induced loss of body weight in *Ae1−/−* mice was greatly increased. *$P < 0.001$ *versus* wild-type.

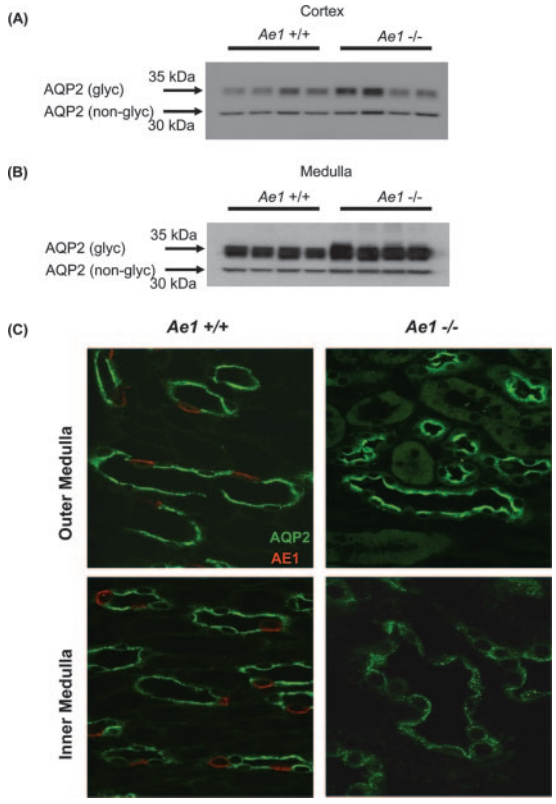

Figure 5. Dysregulation of AQP2 water channel in kidney of AE1-deficient mice. Immunoblots reveal increased AQP2 protein abundance in kidney cortex (A) and medulla (B) of *Ae1−/−* mice in control conditions. (C) Confocal immunofluorescence microscopy of outer medulla (top) shows increased AQP2 immunostaining (green) with strong apical localization in AE1-deficient mice. In contrast, in the inner medulla of *Ae1−/−* kidneys (bottom), AQP2 staining was confined mainly to intracellular vesicular structures, whereas AQP2 in wild-type kidney was predominantly luminal. AE1 staining is in red. Magnification, ×600.

ized to the basolateral membrane of rat and mouse A-IC of the CCD (41), it is an apical protein in type B IC of the rabbit CCD (54) and minimally expressed in OMCD. The Slc26a7 anion exchanger has been reported in the basolateral membrane of A-IC of rat OMCD (37) and in an incompletely defined cell subset in human OMCD (55). Slc26a7-mediated Cl^-/HCO_3^- exchange is activated by hypertonicity (37) (L. Jiang and S.L.A., unpublished observations, 2006), likely in part by mobilization from intracellular stores to the basolateral membrane (56). Slc26a7 is functionally absent in Brattleboro rats and rescued by their treatment with DDAVP (57). The reduced urinary osmolarity of the *slc4a1−/−* mouse suggests that any contribution of Slc26a7 to the residual Cl^-/HCO_3^- activity of the superfused *slc4a1−/−* OMCD might be similarly reduced. However, the localization and the transport activity of mouse Slc26a7 remain controversial, because the protein has been localized with different antibodies to the proximal tubule rather than to A-IC (58) and has been reported to mediate pH-gated Cl^- channel activity rather than Cl^-/HCO_3^- exchange (59).

dRTA as a result of mutations in *SLC4A1* can be inherited as an autosomal recessive or an autosomal dominant disease. dRTA in white patients is generally transmitted in an autosomal dominant pattern (13,15,16), whereas patients of Southeast Asian and Melanesian origin more often present recessive or compound heterozygote forms of the disease (11,12,60). Several mechanisms have been proposed to explain the dominant and recessive forms of dRTA. kAE1 forms functional homodimers or hetero-oligomers, and oligomerization of a dominant negative mutant AE1 with a wild-type polypeptide in the heterozygote A-IC likely explains some dominant disease. The mutant/wild-type heterodimer either fails to traffic to the cell surface or, in some cases, traffics normally but may not function normally at the cell surface (18,61,62). Another subset of kAE1 mutant polypeptides seems to cause dominant dRTA by inappropriate targeting to A-IC apical membrane, with resultant apical bicarbonate secretion likely short-circuiting luminal acid secretion (19,20,63). These interpretations of human dRTA are consistent with the normal renal function displayed by mice that are heterozygous for the *Ae1* null allele. The data strengthen the hypothesis that haploinsufficiency of AE1 does not explain the

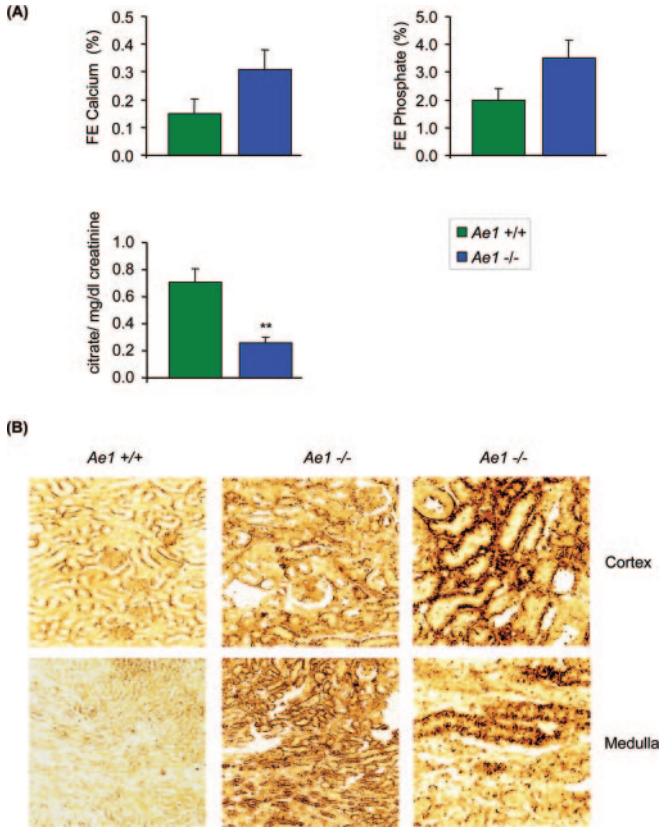

Figure 6. Nephrocalcinosis in kidneys of *Ae1−/−* mice. (A) The fractional excretion (FE %) of calcium and phosphate was increased in acid-loaded AE1-deficient mice, whereas citrate excretion was reduced. $^{**}P < 0.001$. (B) Van Kossa staining for calcium phosphate deposits (brown-black) detected tubular calcification predominantly in the medulla of *Ae1−/−* mice (bottom middle and right). Magnifications: ×100 (left), ×200 (middle), and ×400 (right).

pathophysiology of dominant forms of human dRTA. The reason for the mild concentrating defect that is observed in the heterozygous animals during acid loading remains to be elucidated. This concentrating defect is the only abnormality yet detected in heterozygous mice.

AE1-deficient mice appear as a model for the hydrops fetalis, hemolytic anemia, and dRTA that are exhibited by children who completely lack eAE1 as a result of homozygosity for the autosomal recessive AE1 mutant V488M, Band 3 Coimbra (24). The severe disease in AE1-deficient mice also resembles the hydrops, anemia, and acidosis in Japanese black cattle that are homozygous for AE1 mutations that correspond to human AE1 646X (64). However, most autosomal recessive dRTA in humans is caused by AE1 mutations that are compatible with normal eAE1 abundance and near-normal eAE1 anion transport function in erythrocytes. These conditional loss-of-function mutations in human AE1 are functionally rescued in erythrocytes by the tissue-specific expression of the AE1-binding protein glycophorin A, which likely functions as both trafficking chaperonin and plasmalemmal subunit of eAE1 (10,14).

Patients who have inborn forms of dRTA are often prone to dehydration as a consequence of inappropriate renal water loss (3,15,17,21–23). AE1-deficient mice exhibited a urinary concentrating defect, evident as elevated serum osmolarity with inappropriately low urine osmolality. These mice were unable to increase urine osmolality and decrease urine output during 24-h NH_4Cl loading, leading to severe dehydration as reflected in further elevation of serum osmolarity. This urinary concentrating defect is likely attributable to the intracellular retention of AQP2 water channel polypeptides in the epithelial cells of the inner medullary collecting duct. However, the increased luminal AQP2 expression and total AQP2 abundance in the outer medulla suggest intact regulation by the antidiuretic hormone vasopressin and likely represent compensatory mechanisms. AQP2 in rat inner medullary collecting duct is inhibited by activation of luminal calcium sensing receptor (65). The hypercalciuria of AE1-deficient mice may reduce luminal AQP2 expression *via* stimulation of luminal calcium sensing receptor. Thus, the mild diabetes insipidus of the AE1-deficient mouse kidney may be associated with defective AQP2 regulation that is spatially restricted to the inner medulla.

Nephrocalcinosis and nephrolithiasis can be associated with all forms of complete and incomplete dRTA and often constitute the initial clinical presentation of disease (3,15,23,50). The associated hypercalciuria may reflect osteomalacia or decreased function and/or expression of renal calcium transporting proteins in the presence of metabolic acidosis (66). AE1-deficient mice similarly exhibited hypercalciuria, hypocitraturia, and hyperphosphaturia, and nephrocalcinosis in a predominantly medullary distribution (3,22,51,63,67,68). We additionally noted higher serum urea levels in mice that lacked AE1, suggesting mild renal insufficiency. It remains to be clarified whether the renal failure, which likely has a prerenal component, is secondary to chronic metabolic acidosis, hydronephrosis, nephrocalcinosis, or potentially all of these. Conversely, the mild, chronic renal insufficiency may contribute to the metabolic acidosis.

Conclusion

AE1-deficient mice recapitulate key renal features of inherited forms of dRTA as found in patients with mutations in either AE1 or the ATP6V1B1 and ATP6V0A4 subunits of the vacuolar H^+-ATPase. These mice will allow investigation of the underlying mechanisms of secondary manifestations of the disease such as nephrocalcinosis and dehydration as a result of AQP2 dysregulation. The mice will also allow study of the renal tubular adaptations to congenital dRTA, including upregulation of other bicarbonate transport proteins. AE1-deficient *slc4a1−/−* mice thus demonstrate that renal kAE1 is critical for systemic acid-base homeostasis. kAE1 mediates the essential step in bicarbonate release from acid-secretory IC into blood.

Acknowledgments

This study was supported by grants from the Swiss National Research Foundation (31-68318/02 and 31-109677/1), the 6th EU frame work EuReGene project (005085), and the Hartmann Muller Foundation, Zurich, to C.A.W. and National Institutes of Health grant DK43495 to S.L.A.

Disclosures

None.

References

1. Alper SL: Genetic diseases of acid-base transporters. *Annu Rev Physiol* 64: 899–923, 2002
2. Hamm LL, Alpern RJ: Cellular mechanisms of renal tubular acidification. In: *The Kidney: Physiology and pathophysiology*, 3rd Ed., edited by Seldin DW, Giebisch G, Philadelphia, Lippincott Williams & Wilkins, 2000, pp 1935–1979
3. DuBose TD Jr, Alpern RJ: Renal tubular acidosis. In: *The Metabolic and Molecular Bases of Inherited Disease*, 8th Ed., edited by Scriver CR, Beaudet AL, Sly WS, Valle D, New York, McGraw-Hill, 2001, pp 4983–5021
4. Alper SL: Diseases of mutations in the SLC4A1/AE1 (band 3) Cl^-/HCO_3^- exchanger. In: *Membrane Transporter Diseases*, edited by Broer S, Wagner CA, New York, Kluwer Academic/Plenum Publishers, 2003, pp 39–63
5. Rodriguez-Soriano J: Renal tubular acidosis: The clinical entity. *J Am Soc Nephrol* 13: 2160–2170, 2002
6. Wagner CA, Finberg KE, Breton S, Marshansky V, Brown D, Geibel JP: Renal vacuolar H+-ATPase. *Physiol Rev* 84: 1263–1314, 2004
7. Kollert-Jons A, Wagner S, Hubner S, Appelhans H, Drenckhahn D: Anion exchanger 1 in human kidney and oncocytoma differs from erythroid AE1 in its NH2 terminus. *Am J Physiol* 265: F813–F821, 1993
8. Brosius FC 3rd, Alper SL, Garcia AM, Lodish HF: The major kidney band 3 gene transcript predicts an amino-terminal truncated band 3 polypeptide. *J Biol Chem* 264: 7784–7787, 1989
9. Bruce LJ, Robinson HC, Guizouarn H, Borgese F, Harrison P, King MJ, Goede JS, Coles SE, Gore DM, Lutz HU, Ficarella R, Layton DM, Iolascon A, Ellory JC, Stewart GW: Monovalent cation leaks in human red cells caused by single amino-acid substitutions in the transport domain of the band 3 chloride-bicarbonate exchanger, AE1. *Nat Genet* 37: 1258–1263, 2005
10. Karet FE: Inherited distal renal tubular acidosis. *J Am Soc Nephrol* 13: 2178–2184, 2002
11. Wrong O, Bruce LJ, Unwin RJ, Toye AM, Tanner MJ: Band 3 mutations, distal renal tubular acidosis, and Southeast Asian ovalocytosis. *Kidney Int* 62: 10–19, 2002
12. 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
13. 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
14. Yusoff NM, Van Rostenberghe H, Shirakawa T, Nishiyama K, Amin N, Darus Z, Zainal N, Isa N, Nozu H, Matsuo M: High prevalence of Southeast Asian ovalocytosis in Malays with distal renal tubular acidosis. *J Hum Genet* 48: 650–653, 2003
15. 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
16. Weber S, Soergel M, Jeck N, Konrad M: Atypical distal renal tubular acidosis confirmed by mutation analysis. *Pediatr Nephrol* 15: 201–204, 2000
17. 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 U S A* 95: 6337–6342, 1998
18. Cordat E, Kittanakom S, Yenchitsomanus PT, Li J, Du K, Lukacs GL, Reithmeier RA: Dominant and recessive distal renal tubular acidosis mutations of kidney anion exchanger 1 induce distinct trafficking defects in MDCK cells. *Traffic* 7: 117–128, 2006
19. Devonald MA, Smith AN, Poon JP, Ihrke G, Karet FE: Non-polarized targeting of AE1 causes autosomal dominant distal renal tubular acidosis. *Nat Genet* 33: 125–127, 2003
20. Toye AM, Banting G, Tanner MJ: Regions of human kidney anion exchanger 1 (kAE1) required for basolateral targeting of kAE1 in polarised kidney cells: Mis-targeting explains dominant renal tubular acidosis (dRTA). *J Cell Sci* 117: 1399–1410, 2004
21. Cremers CW, Monnens LA, Marres EH: Renal tubular acidosis and sensorineural deafness: An autosomal recessive syndrome. *Arch Otolaryngol* 106: 287–289, 1980
22. Rodriguez-Soriano J, Vallo A, Castillo G, Oliveros R: Natural history of primary distal renal tubular acidosis treated since infancy. *J Pediatr* 101: 669–676, 1982
23. Santos F, Chan JC: Renal tubular acidosis in children. Diagnosis, treatment and prognosis. *Am J Nephrol* 6: 289–295, 1986
24. Ribeiro ML, Alloisio N, Almeida H, Gomes C, Texier P, Lemos C, Mimoso G, Morle L, Bey-Cabet F, Rudigoz RC, Delaunay J, Tamagnini G: Severe hereditary spherocytosis and distal renal tubular acidosis associated with the total absence of band 3. *Blood* 96: 1602–1604, 2000
25. Peters LL, Shivdasani RA, Liu SC, Hanspal M, John KM, Gonzalez JM, Brugnara C, Gwynn B, Mohandas N, Alper SL, Orkin SH, Lux SE: Anion exchanger 1 (band 3) is required to prevent erythrocyte membrane surface loss but not to form the membrane skeleton. *Cell* 86: 917–927, 1996
26. Bastani B, Purcell H, Hemken P, Trigg D, Gluck S: Expression and distribution of renal vacuolar proton-translocating adenosine triphosphatase in response to chronic acid and alkali loads in the rat. *J Clin Invest* 88: 126–136, 1991
27. Stehberger P, Schulz N, Finberg KE, Karet FE, Giebisch G, Lifton RP, Geibel JP, Wagner CA: Localization and regulation of the ATP6V0A4 (a4) vacuolar H+-ATPase subunit defective in an inherited form of distal renal tubular acidosis. *J Am Soc Nephrol* 14: 3027–3038, 2003
28. Slot C: Plasma creatinine determination. A new and specific Jaffe reaction method. *Scand J Clin Lab Invest* 17: 381–387, 1965
29. Seaton B, Ali A: Simplified manual high performance clinical chemistry methods for developing countries. *Med Lab Sci* 41: 327–336, 1984

30. Berthelot M: Violet d'aniline. *Rep Chim App* 1: 284, 1859
31. Chan JC: The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. *Clin Biochem* 5: 94–98, 1972
32. Southgate CD, Chishti AH, Mitchell B, Yi SJ, Palek J: Targeted disruption of the murine erythroid band 3 gene results in spherocytosis and severe haemolytic anaemia despite a normal membrane skeleton. *Nat Genet* 14: 227–230, 1996
33. Hays SR: Mineralocorticoid modulation of apical and basolateral membrane H+/OH−/HCO3− transport processes in the rabbit inner stripe of outer medullary collecting duct. *J Clin Invest* 90: 180–187, 1992
34. Alper SL, Chernova MN, Williams J, Zasloff M, Law FY, Knauf PA: Differential inhibition of AE1 and AE2 anion exchangers by oxonol dyes and by novel polyaminosterol analogs of the shark antibiotic squalamine. *Biochem Cell Biol* 76: 799–806, 1998
35. Alper SL, Natale J, Gluck S, Lodish HF, Brown D: Subtypes of intercalated cells in rat kidney collecting duct defined by antibodies against erythroid band 3 and renal vacuolar H+-ATPase. *Proc Natl Acad Sci U S A* 86: 5429–5433, 1989
36. Alper SL, Stuart-Tilley AK, Biemesderfer D, Shmukler BE, Brown D: Immunolocalization of AE2 anion exchanger in rat kidney. *Am J Physiol* 273: F601–F614, 1997
37. Petrovic S, Barone S, Xu J, Conforti L, Ma L, Kujala M, Kere J, Soleimani M: SLC26A7: A basolateral Cl−/HCO3− exchanger specific to intercalated cells of the outer medullary collecting duct. *Am J Physiol Renal Physiol* 286: F161–F169, 2003
38. Eladari D, Blanchard A, Leviel F, Paillard M, Stuart-Tilley AK, Alper SL, Podevin RA: Functional and molecular characterization of luminal and basolateral Cl−/HCO3− exchangers of rat thick limbs. *Am J Physiol* 275: F334–F342, 1998
39. Stuart-Tilley AK, Shmukler BE, Brown D, Alper SL: Immunolocalization and tissue-specific splicing of AE2 anion exchanger in mouse kidney. *J Am Soc Nephrol* 9: 946–959, 1998
40. Frische S, Zolotarev AS, Kim YH, Praetorius J, Alper S, Nielsen S, Wall SM: AE2 isoforms in rat kidney: Immunohistochemical localization and regulation in response to chronic NH4Cl loading. *Am J Physiol Renal Physiol* 286: F1163–F1170, 2004
41. Ko SBH, Xiang L, Hager H, Rojek A, Choi JY, Licht C, Suzuki M, Muallem S, Nielsen S, Ishibashi K: AE4 is a DIDS-Sensitive Cl−/HCO3− exchanger in the basolateral membrane of the rat and mouse renal CCD and the SMG duct. *Am J Physiol Cell Physiol* 283: C1206–C1218, 2002
42. Frische S, Kwon TH, Frokiaer J, Madsen KM, Nielsen S: Regulated expression of pendrin in rat kidney in response to chronic NH4Cl or NaHCO3 loading. *Am J Physiol Renal Physiol* 284: F584–F593, 2003
43. 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 U S A* 98: 4221–4226, 2001
44. Wagner CA, Finberg KE, Stehberger PA, Lifton RP, Giebisch GH, Aronson PS, Geibel JP: Regulation of the expression of the Cl−/anion exchanger pendrin in mouse kidney by acid-base status. *Kidney Int* 62: 2109–2117, 2002
45. Wall SM, Hassell KA, Royaux IE, Green ED, Chang JY, Shipley GL, Verlander JW: Localization of Pendrin in mouse kidney. *Am J Physiol Renal Physiol* 284: F229–F241, 2002
46. Petrovic S, Wang Z, Ma L, Soleimani M: Regulation of the apical Cl−/HCO−3 exchanger pendrin in rat cortical collecting duct in metabolic acidosis. *Am J Physiol Renal Physiol* 284: F103–F112, 2003
47. Yasui M, Hazama A, Kwon TH, Nielsen S, Guggino WB, Agre P: Rapid gating and anion permeability of an intracellular aquaporin. *Nature* 402: 184–187, 1999
48. Boettger T, Hubner CA, Maier H, Rust MB, Beck FX, Jentsch TJ: Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. *Nature* 416: 874–878, 2002
49. Vincourt JB, Jullien D, Amalric F, Girard JP: Molecular and functional characterization of SLC26A11, a sodium-independent sulphate transporter from high endothelial venules. *FASEB J* 17: 890–892, 2003
50. Peces R: Long-term follow-up in distal renal tubular acidosis with sensorineural deafness. *Pediatr Nephrol* 15: 63–65, 2000
51. Aruga S, Wehrli S, Kaissling B, Moe OW, Preisig PA, Pajor AM, Alpern RJ: Chronic metabolic acidosis increases NaDC-1 mRNA and protein abundance in rat kidney. *Kidney Int* 58: 206–215, 2000
52. Finberg KE, Wagner CA, Bailey MA, Paunescu TG, Breton S, Brown D, Giebisch G, Geibel JP, Lifton RP: The B1 subunit of the H+ATPase is required for maximal urinary acidification. *Proc Natl Acad Sci U S A* 102: 13616–13621, 2005
53. 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 B1 subunit of H+-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet* 21: 84–90, 1999
54. 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 HCO3− transporter superfamily is an apical anion exchanger of beta-intercalated cells in the kidney. *J Biol Chem* 276: 8180–8189, 2001
55. Lohi H, Kujala M, Makela S, Lehtonen E, Kestila M, Saarialho-Kere U, Markovich D, Kere J: Functional characterization of three novel tissue-specific anion exchangers SLC26A7, -A8, and -A9. *J Biol Chem* 277: 14246–14254, 2002
56. Xu J, Worrell RT, Li HC, Barone SL, Petrovic S, Amlal H, Soleimani M: Chloride/bicarbonate exchanger SLC26A7 is localized in endosomes in medullary collecting duct cells and is targeted to the basolateral membrane in hypertonicity and potassium depletion. *J Am Soc Nephrol* 17: 956–967, 2006
57. Petrovic S, Amlal H, Sun X, Karet F, Barone S, Soleimani M: Vasopressin induces expression of the Cl−/HCO3− exchanger SLC26A7 in kidney medullary collecting ducts of Brattleboro rats. *Am J Physiol Renal Physiol* 290: F1194–F1201, 2006
58. Dudas PL, Mentone S, Greineder CF, Biemesderfer D, Aronson PS: Immunolocalization of anion transporter

Slc26a7 in mouse kidney. *Am J Physiol Renal Physiol* 290: F937–F945, 2006
59. Kim KH, Shcheynikov N, Wang Y, Muallem S: SLC26A7 is a Cl− channel regulated by intracellular pH. *J Biol Chem* 280: 6463–6470, 2005
60. 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
61. 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–F820, 2002
62. Dahl NK, Jiang L, Chernova MN, Stuart-Tilley AK, Shmukler BE, Alper SL: Deficient HCO3− transport in an AE1 mutant with normal Cl− transport can be rescued by carbonic anhydrase II presented on an adjacent AE1 protomer. *J Biol Chem* 278: 44949–44958, 2003
63. Rungroj N, Devonald MA, Cuthbert AW, Reimann F, Akkarapatumwong V, Yenchitsomanus PT, Bennett WM, Karet FE: A novel missense mutation in AE1 causing autosomal dominant distal renal tubular acidosis retains normal transport function but is mistargeted in polarized epithelial cells. *J Biol Chem* 279: 13833–13838, 2004
64. Inaba M, Yawata A, Koshino I, Sato K, Takeuchi M, Takakuwa Y, Manno S, Yawata Y, Kanzaki A, Sakai J, Ban A, Ono K, Maede Y: Defective anion transport and marked spherocytosis with membrane instability caused by hereditary total deficiency of red cell band 3 in cattle due to a nonsense mutation. *J Clin Invest* 97: 1804–1817, 1996
65. Sands JM, Naruse M, Baum M, Jo I, Hebert SC, Brown EM, Harris HW: Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited water permeability in rat kidney inner medullary collecting duct. *J Clin Invest* 99: 1399–1405, 1997
66. Nijenhuis T, Renkema KY, Hoenderop JG, Bindels RJ: Acid-base status determines the renal expression of Ca2+ and Mg2+ transport proteins. *J Am Soc Nephrol* 17: 617–626, 2006
67. McSherry E: Renal tubular acidosis in childhood. *Kidney Int* 20: 799–809, 1981
68. Nicoletta JA, Schwartz GJ: Distal renal tubular acidosis. *Curr Opin Pediatr* 16: 194–198, 2004

Supplemental information for this article is available online at http://www.jasn.org/.