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Acute regulated expression of pendrin in human urinary exosomes

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Abstract It is well known that pendrin, an apical Cl^-/HCO_3^- exchanger in type B intercalated cells, is modulated by chronic acid-base disturbances and electrolyte intake. To study this adaptation further at the acute level, we analyzed urinary exosomes from individuals subjected to oral acute acid, alkali, and NaCl loading. Acute oral NH_4Cl loading (n = 8) elicited systemic acidemia with a drop in urinary pH and an increase in urinary NH_4 excretion. Nadir urinary pH was achieved 5 h after NH_4Cl loading. Exosomal pendrin abundance was dramatically decreased at 3 h after acid loading. In contrast, after acute equimolar oral $NaHCO_3$ loading (n = 8), urinary and venous blood pH rose rapidly with a significant attenuation of urinary NH_4 excretion. Alkali loading caused rapid upregulation of exosomal pendrin abundance at 1 h and normalized within 3 h of treatment. Equimolar NaCl loading (n = 6) did not alter urinary or venous blood pH or urinary NH_4 excretion. However, pendrin abundance in urinary exosomes was significantly reduced at 2 h of NaCl ingestion with lowest levels observed at 4 h after treatment. In patients with inherited distal renal tubular acidosis (dRTA), pendrin abundance in urinary exosomes was greatly reduced and did not change upon oral NH_4Cl loading. In summary, pendrin can be detected and quantified in human urinary exosomes by immunoblotting. Acid, alkali, and NaCl loadings cause acute changes in pendrin abundance in urinary exosomes within a few hours. Our data suggest that exosomal pendrin is a promising urinary biomarker for acute acid-base and volume status changes in humans.

Keywords Pendrin · SLC26A4 · Acid-base homeostasis · Distal renal tubular acidosis · Urinary exosomes

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Introduction

Pendrin (SLC26A4) was originally identified as the gene mutated in patients with Pendred syndrome, a genetic disorder associated with deafness and goiter (OMIM 274600) [6]. Functional studies in Xenopus oocytes later revealed that pendrin acts as an imperative exchanger for various anions including bicarbonate, chloride, iodide, and formate [32, 33]. Apart from inner ear [4] and thyroid [24], pendrin is expressed in the apical membranes of type B intercalated cells of the renal late distal convoluted tubule (DCT2), connecting tubule (CNT), and cortical collecting duct (CCD) [25]. Type A intercalated cells, which are endowed with an apical V-ATPase (vacuolar-type H^+-ATPase) and basolateral Cl^-/HCO_3^- exchanger, secrete protons and release bicarbonate into blood [3, 28–30]. Secretion of HCO_3^- is carried out by type

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B intercalated cells which have basolateral V-ATPase and the Cl^-/HCO_3^- exchanger pendrin at the apical membrane [25, 26, 28, 41]. Royaux et al. have shown that luminal Cl^-/HCO_3^- exchanger activity was absent from type B intercalated cells in CCDs isolated from pendrin KO mice [25].

Experimental acute acidosis in rabbits leads to increased proton secretion and decreased HCO_3^- secretion, respectively, by type A and B intercalated cells in isolated perfused CCDs [27, 29, 36]. At least part of these acute adaptive changes occurs by downregulation of pendrin at the apical plasma membrane [31]. After a 3-day period of metabolic acidosis, pendrin messenger RNA (mRNA), protein abundance, and a number of pendrin-positive cells in rabbit kidneys were found to be decreased [21]. Similar results were obtained in mice and rats subjected to chronic metabolic acidosis for a week [8, 18, 42]. On the contrary, metabolic alkalosis induced by chronic $NaHCO_3$ administration for a week caused increased pendrin protein expression in rodents [8, 42]. Upon alkali treatment, renal pendrin expression was found to normalize within 12–18 h in acidotic rabbits [21]. Pendrin expression is also modulated by NaCl intake, mineralocorticoids, and particularly by urinary Cl^- excretion [11, 43]. Thus, pendrin is involved not only in acid-base maintenance but also in volume homeostasis. Results obtained in animals are difficult to extrapolate to human physiology because of significant differences in dietary habits between rodents and humans. Therefore, the functional relevance of pendrin in the human kidney remains unsettled.

One of the most common congenital acid-base disorders is distal renal tubular acidosis (dRTA) characterized by hyperchloremia, metabolic acidosis with reduced net acid secretion due to inability to lower urinary pH [13, 23]. The incomplete form of dRTA (i.e., alkaline urine, but absence of systemic acidosis) is diagnosed by the oral NH_4Cl loading test [45]. Recently we found that the B1 subunit of the V-ATPase is upregulated in urinary exosomes after acute NH_4Cl loading (Pathare, manuscript submitted). However, nothing is known about the acute response of type B intercalated cells or pendrin expression in the humans following acute acid or alkali challenges. The presence of pendrin in the apical membrane of type B intercalated cells and its pivotal role in the chronic acid-base adaptation raises the important question about the rapidity of the events in pendrin regulation or its apical abundance.

We hypothesized that acute systemic acidosis/alkalosis induced in humans by NH_4Cl or $NaHCO_3$ loading would affect pendrin expression. The specific aim was to investigate whether and how fast regulation occurs after an acute acid or base loading. To this end, we isolated and analyzed urinary exosomes from healthy individuals subjected to oral NH_4Cl, $NaHCO_3$, or NaCl loading. Furthermore, we show differential pendrin regulation in urinary exosomes isolated from dRTA patients compared to healthy subjects.

Materials and methods

Study participants, patients, and test procedures

All study participants (males, aged 25–45 years) gave written informed consent, and the study protocol was approved by the ethics commission of the canton of Berne. Participants underwent the classical ("short") NH_4Cl loading test [45] and equimolar $NaHCO_3$ or NaCl loading on separate days after overnight fasting and with at least 3 weeks between tests. All tests started at 0800 h; venous blood samples were obtained for blood chemistry, pH, and blood gases at 0800, 1000, and 1200 h. Urine was collected hourly from 0800 to 1400 h. Venous blood gas and electrolyte analysis was performed immediately after collection on a ABL800FLEX blood gas analyzer (Radiometer, Thalwil, Switzerland). Urine pH was measured by a S20 SevenEasy pH meter (Mettler Toledo, Greifensee, Switzerland) immediately after collection. For the NH_4Cl loading test, oral NH_4Cl at a dose of 100 mg/kg (1.87 mmol/kg) body weight was given. For $NaHCO_3$ loading, $NaHCO_3$ at a dose of 157 mg/kg (1.87 mmol/kg) was given. NaCl was ingested at a dose of 110 mg/kg (1.87 mmol/kg). Study participants were recommended to drink 200 ml of water hourly during tests. Protease inhibitor cocktail tablets (Roche, Mannheim, Germany) were added immediately after urine collection. Samples were stored at − 80 °C until use. The information about the dRTA patients included in the study is summarized in Table 1.

Measurement of urinary parameters

Urine samples were aliquoted and sent to the Central Laboratory of the University Hospital of Bern, Switzerland for determination of urinary electrolytes (Na, K, Cl) and creatinine. Urinary ammonium was measured using the Berthelot method [2]. Urinary osmolality was measured on a Vapro 5600 (Wescor, Logan, UT) vapor pressure osmometer.

Urinary exosomes

Exosomes were isolated according to a previously established and refined protocol yielding highly pure exosomal membranes without significant contamination of non-exosomal proteins [7, 19]. Twenty milliliters of collected urine was centrifuged at 17,000×*g* for 15 min at 24 °C in an Ultra Centrifuge (Beckman Coulter, CA, USA) with a TFT70.38 rotor. The supernatant was removed and incubated at room temperature for 25 min. The pellet was resuspended in 200 μl isolation solution (250 mmol/l sucrose and 10 mmol/l triethanolamine-HCl, pH 7.6) and 50 μl 3.24 mol/l dithiothreitol (DTT) and subsequently centrifuged at 17,000×*g* for 15 min at 24 °C. This supernatant was collected and combined with supernatant obtained from the previous step and centrifuged at

Table 1 Genetic and laboratory characteristics of dRTA patients

Nr	Sex	Age (years)		Plasma Na (mmol/l)	Plasma K (mmol/l)	Plasma Cl (mmol/l)	Plasma HCO_3 (mmol/l)
1	F	30	*ATP6V1B1*, homozygous (c.242T>C; p.Leu81Pro)	141	3.4	110	23.1
2	F	26	*ATP6V1B1*, compound heterozygous (c.242T>C; p.Leu81Pro and c.1037C>G; p.Pro346Arg)	137	3.3	112	20.3
3	M	45	*ATP6V0A4*, homozygous (c.2257C>T; p.GLn753)	138	4.1	109	21.4
4	M	21	*ATP6V0A4*, homozygous (c.1185delC; p.Tyr396Thrfs*12)	138	3.3	111	22.2
5	M	23	*ATP6V1B1*, homozygous (c.242T>C; p.Leu81Pro)	142	3.6	117	19.9

200,000×*g* for 2 h at 24 °C. The exosome pellet was dissolved in 50 μl of Laemmli buffer (0.6% *w/v* SDS, 3% *v/v* glycerol, 18 mmol/l Tris-HCl pH 6.8 and 0.003% *w/v* bromophenol blue) and stored at − 20 °C for further use.

Preparation of mouse kidney lysates

Generation and breeding of pendrin KO mice were described previously [1]. Wild-type (WT) and pendrin KO mice kidneys were removed and immediately shock-frozen in liquid nitrogen. Kidneys of WT and pendrin KO mice were kindly provided by Prof. M. Soleimani, Center on Genetics of Transport and Epithelial Biology and Dept. of Internal Medicine, University of Cincinnati, OH, USA. Renal tissue was homogenized with an electric homogenizer at 4 °C in lysis buffer (54.6 mM HEPES; 2.69 mM $Na_4P_2O_7$; 360 mM NaCl; 10% [vol/vol] glycerol; 1% [vol/vol] NP40) containing protease inhibitors (Roche, Mannheim, Germany). Homogenates were clarified by centrifugation at 20,000×*g* for 20 min and subsequently used for SDS-PAGE and immunoblotting.

Immunoblotting and antibodies

Urinary exosomal pellets resuspended in Laemmli buffer were incubated at 60 °C for 15 min. The volume of urinary exosomes suspension per lane was adjusted according to the urinary creatinine concentration and loaded on 8% *v/v* gels for protein separation. Proteins were subsequently transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore Corporation, Bedford, MA, USA) and then used for immunoblotting with primary antibodies. The following primary antibodies were used: Rabbit polyclonal pendrin at 1:2000 dilutions [11] and mouse polyclonal anti-alix (Abcam, Cat no.: ab88743) at 1:500 dilutions. Secondary antibodies used were HRP-conjugated anti-rabbit (Sigma-Aldrich, St. Louis, MO, USA; 1:20,000 dilution) and anti-mouse (Sigma-Aldrich, St. Louis, MO, USA; 1:3000 dilution). Image quantification was performed by the ImageJ software.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Comparisons were made between groups using either Student's *t* test or one-way ANOVA with posthoc Tukey's analysis, as appropriate. All statistical tests were two sided. A p value of < 0.05 was considered statistically significant. Data were analyzed using Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA).

Results

Pendrin is expressed in human urinary exosomes

At least in theory, human urinary exosomes contain apical proteins present in the epithelium along the nephron and collecting duct system [9]. In a first step, we detected pendrin in urinary exosomes isolated from second morning spot urine from three healthy subjects. A previously characterized antibody raised against the C-terminal region of rat pendrin was used to test for pendrin expression in human urinary exosomes [11]. As shown in Fig. 1, a ~ 110 kDa band was detected in urinary exosomes, corresponding to the expected molecular mass of pendrin. Using total crude mouse kidney lysates, immunoblotting showed a similar size band in exosomes lanes. Importantly, no band was detected in lysates from pendrin KO kidneys. Ponceau staining confirmed equal loading for all samples.

Downregulation of pendrin in urinary exosomes after NH_4Cl loading

Results of acute NH_4Cl loading are depicted in Fig. 2. All participants acidified their urine to a pH < 5.3, which is considered a normal response [45] (Fig. 2b). Nadir urinary pH was achieved at 5 h, which is comparable to previous studies [5, 44]. Venous blood pH and bicarbonate, measured at baseline and at 2 and 4 h, respectively, revealed the presence of a significant metabolic acidosis at 2 h with slight recovery after 4 h (Fig. 2a). Urinary ammonium excretion increased

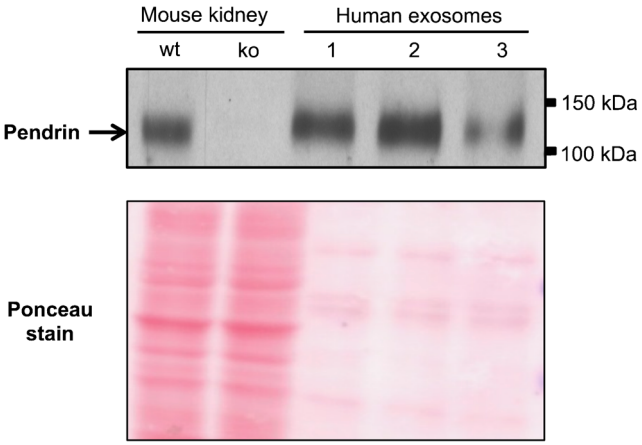

Fig. 1 Specificity of the pendrin antibody and detection of pendrin in human urinary exosomes by immunoblotting. Upper panel: Kidney lysates of WT (lane 1) and pendrin KO mice (lane 2). Lanes 3–5: urinary exosomes isolated from three healthy individuals, blotted with pendrin antibody. Lower panel: Ponceau staining as loading control

significantly at 2, 5, and 6 h (Fig. 2b). Urinary sodium excretion increased slightly, but significantly at 3 h, but we observed no changes in urinary potassium excretion (Fig. 2c). As expected, urinary chloride excretion rose significantly at 2 h after NH_4Cl ingestion (Fig. 2c). Urinary creatinine concentration and urinary osmolality were not significantly different throughout the experiment (Supplemental Fig. S2). Figure 3a shows two representative immunoblots of exosomes isolated from NH_4Cl-loaded participants, probed for pendrin and alix. The exosomal pendrin abundance was normalized to the exosomal housekeeping protein alix [16, 19] and compared to baseline. Pooled analysis of eight individual tests revealed an acute and sustained downregulation of pendrin abundance in urinary exosomes upon acid loading (Supplemental Fig. S1). Densitometric analysis showed that after 3 h the pendrin abundance was significantly lower compared to baseline and remained lower throughout the entire experimental period (Fig. 3b).

Rapid upregulation of pendrin in urinary exosomes after $NaHCO_3$ loading

In a next step, an equimolar oral alkali challenge with $NaHCO_3$ was administered to all participants. As shown in Fig. 4b, urinary pH rose rapidly during $NaHCO_3$ loading along with venous blood pH and bicarbonate (Fig. 4a), indicating the presence of a systemic metabolic alkalosis. Urinary ammonium excretion decreased compared to baseline and was significantly lower at 3, 4, and 5 h (Fig. 4b). Urinary sodium and potassium excretion transiently rose after $NaHCO_3$ administration, while chloride excretion dropped (Fig. 4c).

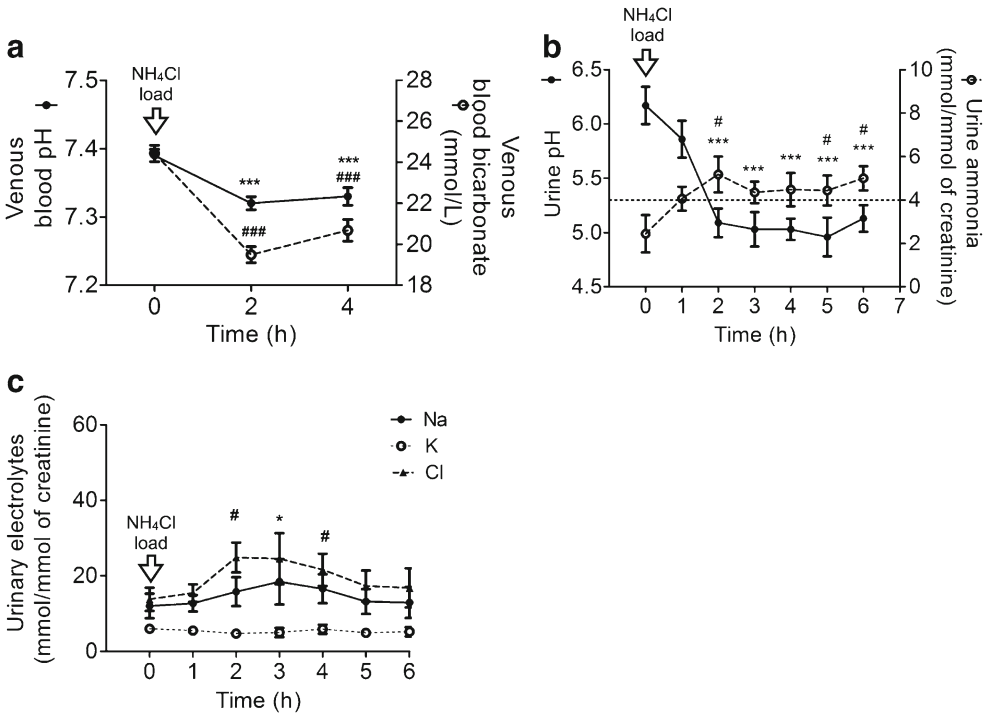

Fig. 2 Blood and urinary parameters during NH_4Cl loading. Time 0 represents baseline (prior to NH_4Cl ingestion). Arithmetic means ± SEM of different parameters (n = 8). **a** Venous blood pH and bicarbonate. Asterisk and number sign indicate comparison of pH and bicarbonate, respectively, with reference to baseline. **b** Urine pH and ammonia excretion. Asterisk and number sign indicate comparison of pH and ammonia respectively with reference to baseline. **c** Urinary Na, K, Cl excretion. Asterisk and number sign indicate comparison of Na and Cl excretion, respectively, with reference to baseline. Urinary K excretion was not found significantly different during the course of experiment. Data are means ± SEM; n = 5. $^{*,\#}P < 0.05$, $^{**,\#\#}P < 0.01$, $^{***,\#\#\#}P < 0.001$ compared to baseline

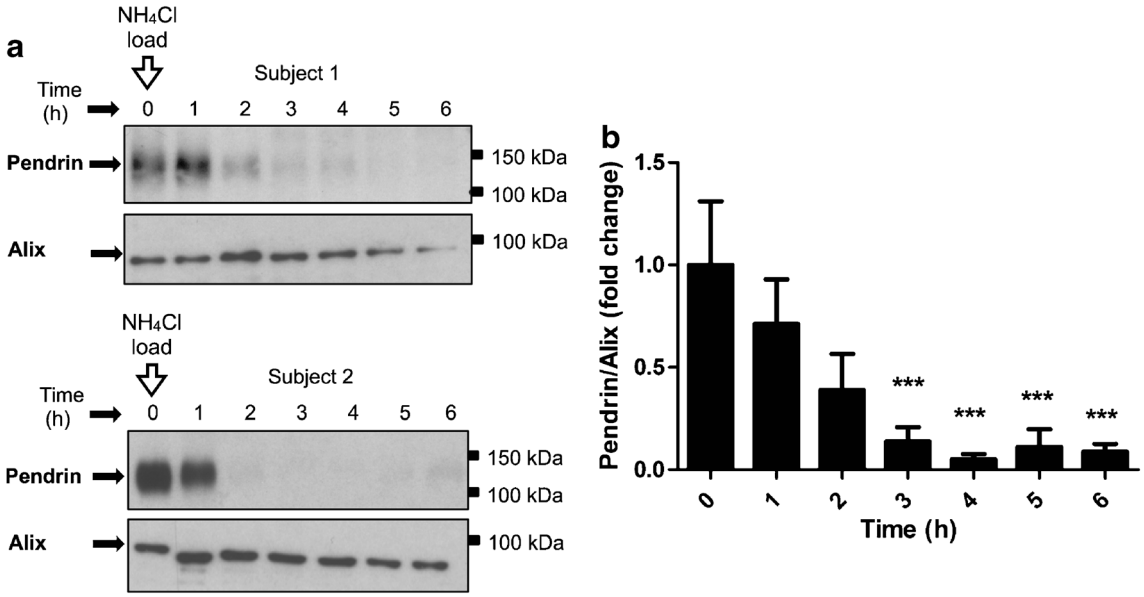

Fig. 3 Effect of NH_4Cl loading on pendrin abundance in urinary exosomes. **a** Representative immunoblots of urinary exosomes isolated from two participants, probed with pendrin (upper panel) or alix (lower panel) antibodies. **b** Quantification of immunoblots of eight participants. All data were normalized to the respective baseline. Data are means ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to baseline

Urinary creatinine concentration and osmolality remained unaltered throughout the experiment (Supplemental Fig. S4). Urinary exosomes from hourly collected urine samples of all participants were isolated, immunoblotted and probed for pendrin and alix. Figure 5a shows representative immunoblots of two participants subjected to acute alkali loading. Pooled analysis of eight subjects demonstrated a rapid increase in pendrin abundance in urinary exosomes after 1–2 h of $NaHCO_3$ loading (Supplemental Fig. S3). Densitometric analysis showed that highest levels of pendrin abundance in exosomes were observed at 1 h (Fig. 5). Pendrin abundance returned to baseline after 3 h of $NaHCO_3$ loading and remained constant thereafter.

Pendrin abundance in urinary exosomes is markedly reduced after an acute NaCl load

To analyze the effect of an acute chloride load on pendrin abundance, which might have played a role in NH_4Cl induced pendrin regulation, we administered an oral equimolar NaCl solution to healthy subjects. Urinary pH, ammonia, venous blood pH, and bicarbonate were unchanged throughout the experiment (Fig. 6a, b). As expected, excretion of urinary electrolytes was transiently and dramatically increased after NaCl ingestion (Fig. 6c). Urinary creatinine concentration and osmolality remained unaltered throughout the experiment (Supplemental Fig. S6).

Immunoblotting of urinary exosomes isolated from hourly collected urine samples of all participants were immunoblotted and probed for pendrin and alix. Figure 7a shows immunoblots of two representative subjects that underwent the NaCl loading test. In total, immunoblots were obtained from six healthy individuals and used for densitometric analysis (Supplemental Fig. S5). As shown in Fig. 7b, levels of pendrin in urinary exosomes were gradually and significantly decreased from 2 to 4 h. The lowest levels were observed 4 h after NaCl loading.

Pendrin abundance in urinary exosomes is greatly reduced in dRTA patients

Patients with inherited forms of dRTA showed significantly lower venous blood bicarbonate levels when compared to healthy subjects (Fig. 8b). As depicted in Fig. 8a, despite of a lower venous blood pH in dRTA patients, the difference did not reach statistical significance ($p = 0.067$). Alkali therapy in dRTA patients was stopped the day before the experiment. The second morning spot urine samples were collected from three dRTA patients and corresponding healthy subjects. Urinary exosomes were isolated and immunoblotted for pendrin and alix as described earlier. Figure 8c shows decreased pendrin levels in urinary exosomes of dRTA patients compared to healthy subjects. Further quantification showed significantly reduced pendrin levels in dRTA patients compared to healthy subjects (Fig. 8d).

Effect of NH_4Cl loading on pendrin abundance in dRTA patients

Five patients with inherited dRTA were recruited to isolate urinary exosomes followed by NH_4Cl loading test; alkali therapy was stopped the day before the acid loading experiment.

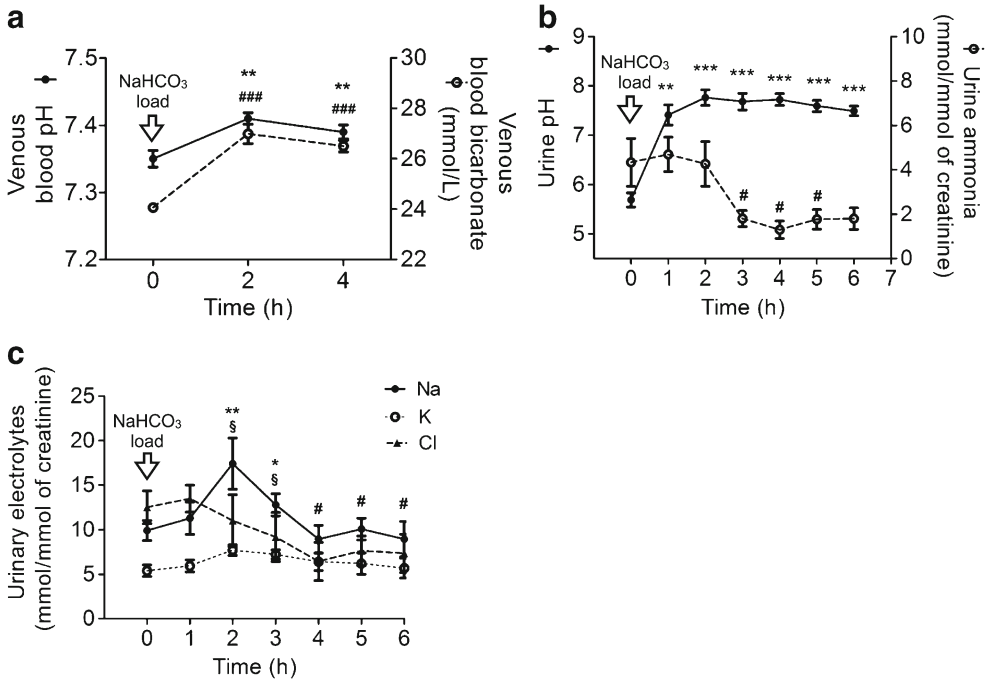

Fig. 4 Blood and urinary parameters during $NaHCO_3$ loading. Time 0 represents baseline (prior to $NaHCO_3$ ingestion). Arithmetic means ± SEM of different parameters (n = 8). **a** Venous blood pH and bicarbonate. Asterisk and number sign indicate comparison of pH and bicarbonate, respectively, with reference to baseline. **b** Urine pH and ammonia excretions. Asterisk and number sign indicate comparison of pH and ammonia, respectively, with reference to baseline. **c** Urinary Na, K, and Cl excretions. Asterisk, number sign, and section sign indicate comparison of Na, Cl, and K excretion, respectively, with reference to baseline. Urinary K excretion was not found significantly different during the course of experiment. Data are means ± SEM; n = 5. $^{*,\#,\S}P < 0.05$, $^{**,\#\#}P < 0.01$, $^{***,\#\#\#}P < 0.001$ compared to baseline

The information about the patients is summarized in a Table 1. Figure 9a shows venous blood pH and bicarbonate levels after acid loading. As shown in Fig. 9b, patients had alkaline urine and their urinary pH was unresponsive to acute acid loading. Urinary ammonia excretion increased in the first 2 h after acid loading, but the increase did not reach statistical significance due to a large interindividual variability. Urinary electrolyte excretion was not significantly altered throughout the experiment and urinary osmolality and creatinine levels remained unchanged (Fig. 9b; Supplemental Fig. S8). Figure 10a

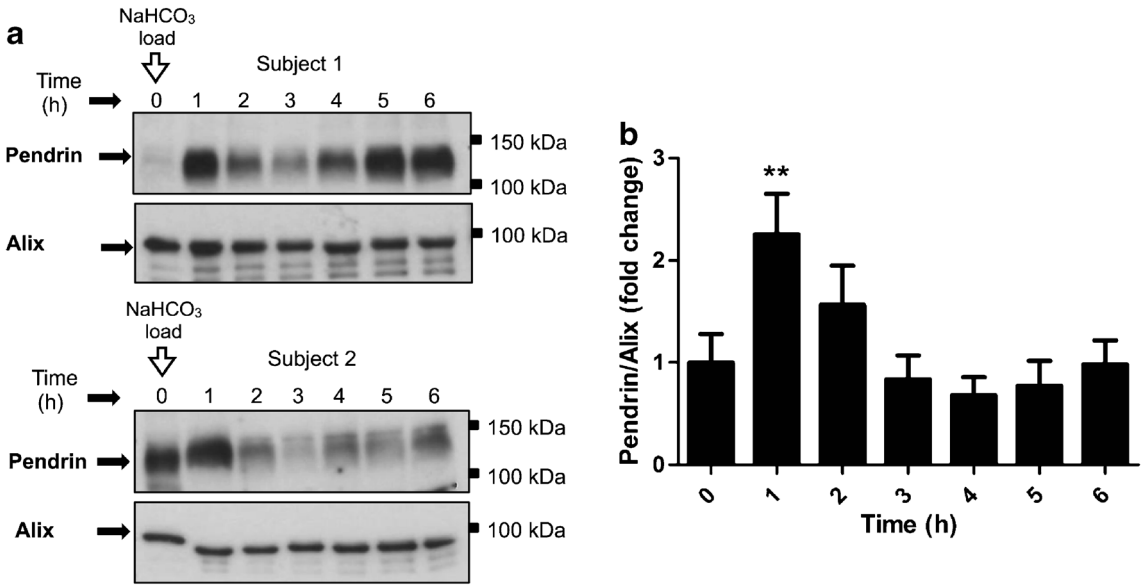

Fig. 5 Effect of $NaHCO_3$ on pendrin abundance in urinary exosomes. **a** Representative immunoblots of urinary exosomes isolated from two participants, probed with pendrin (upper panel) or alix (lower panel) antibodies. **b** Quantification of immunoblots of eight participants. All data were normalized to the respective baseline. Data are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to baseline

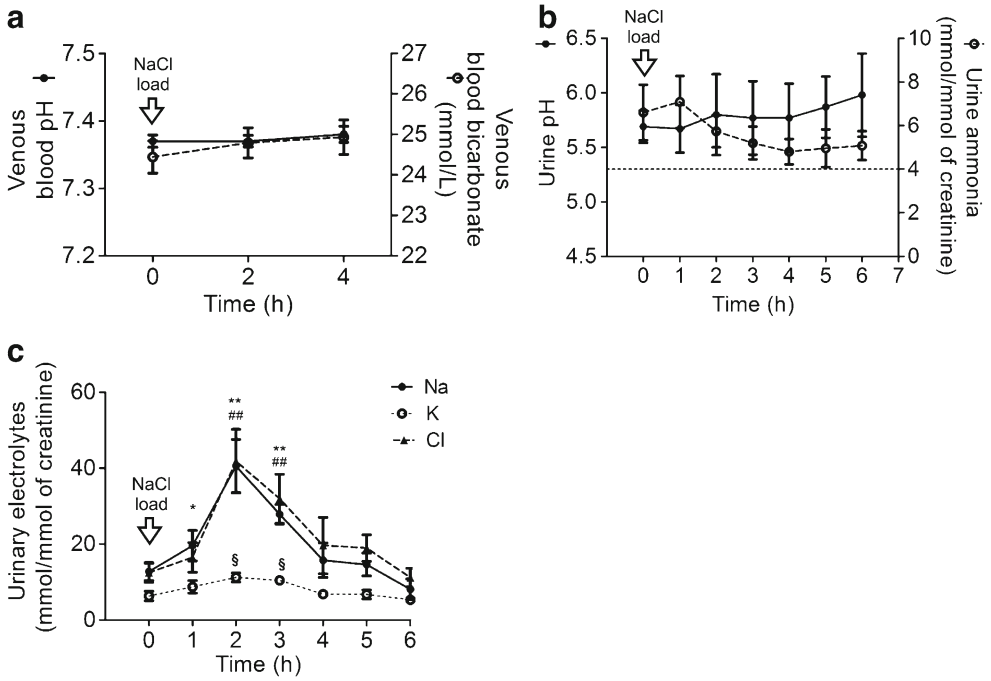

Fig. 6 Blood and urinary parameters during NaCl loading. Time 0 represents baseline (prior to NaCl ingestion). Arithmetic means ± SEM of different parameters (n = 6). **a** Venous blood pH and bicarbonate indicate comparison of pH and bicarbonate respectively with reference to baseline. **b** Urine pH and ammonia excretion indicate comparison of pH and ammonia respectively with reference to baseline. **c** Urinary Na, K, and Cl excretions. Asterisk, number sign, and section sign indicate comparison of Na, Cl, and K excretions, respectively, with reference to baseline. Urinary K excretion was not found significantly different during the course of experiment. Data are means ± SEM; n = 5. $^{*,\#,\S}P < 0.05$, $^{**,\#\#}P < 0.01$, $^{***,\#\#\#}P < 0.001$ compared to baseline

represents immunoblots of pendrin and alix from two dRTA patients that had undergone NH_4Cl loading. Densitometric analysis of all five tests in dRTA patients showed that pendrin levels in urinary exosomes were not significantly altered upon acute NH_4Cl loading (Fig. 10b; Supplemental Fig. 7).

Discussion

The present study focuses on the abundance of pendrin in urinary exosomes isolated from healthy subjects and patients with inherited dRTA and its regulation by well-defined acute acid-

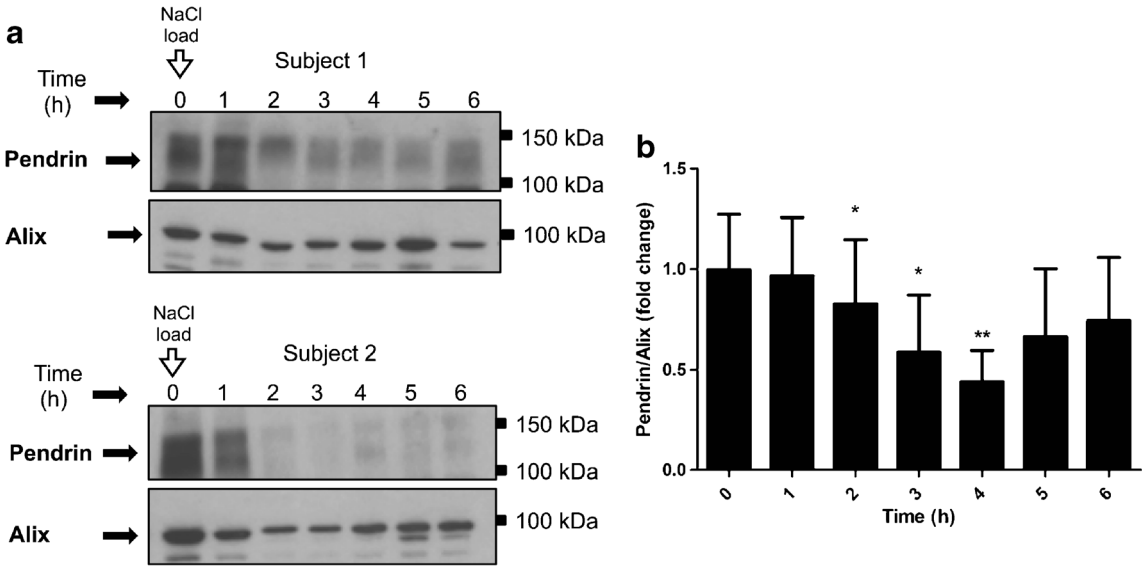

Fig. 7 Effect of NaCl on pendrin abundance in urinary exosomes. **a** Representative immunoblots of urinary exosomes isolated from two participants, probed with pendrin (upper panel) or alix (lower panel) antibodies. **b** Quantification of immunoblots of six participants. All data were normalized to the respective baseline. Data are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to baseline

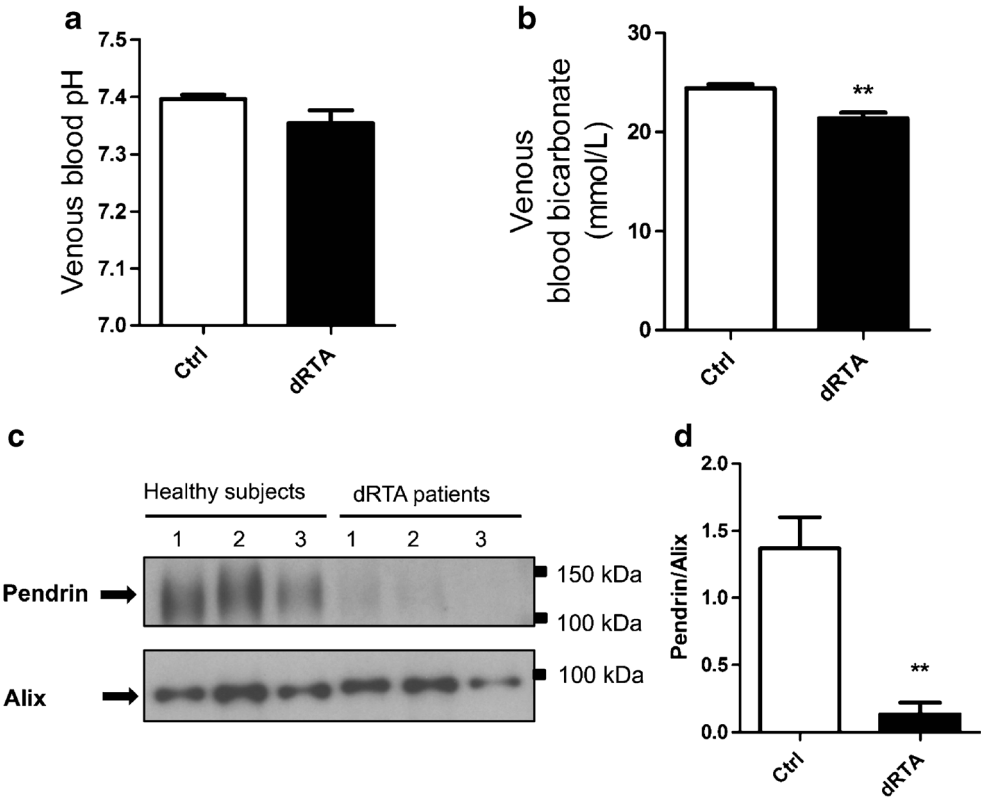

Fig. 8 Metabolic acidosis and pendrin levels in patients with inherited dRTA. **a** Venous blood pH. **b** Venous blood bicarbonate levels in dRTA patients ($n = 5$). **c** Immunoblots of urinary exosomes of patients and healthy subjects, probed with pendrin (upper panel) and alix (lower panel) antibodies. **d** Quantification of immunoblots. All data were normalized to the respective baseline. Data are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to baseline

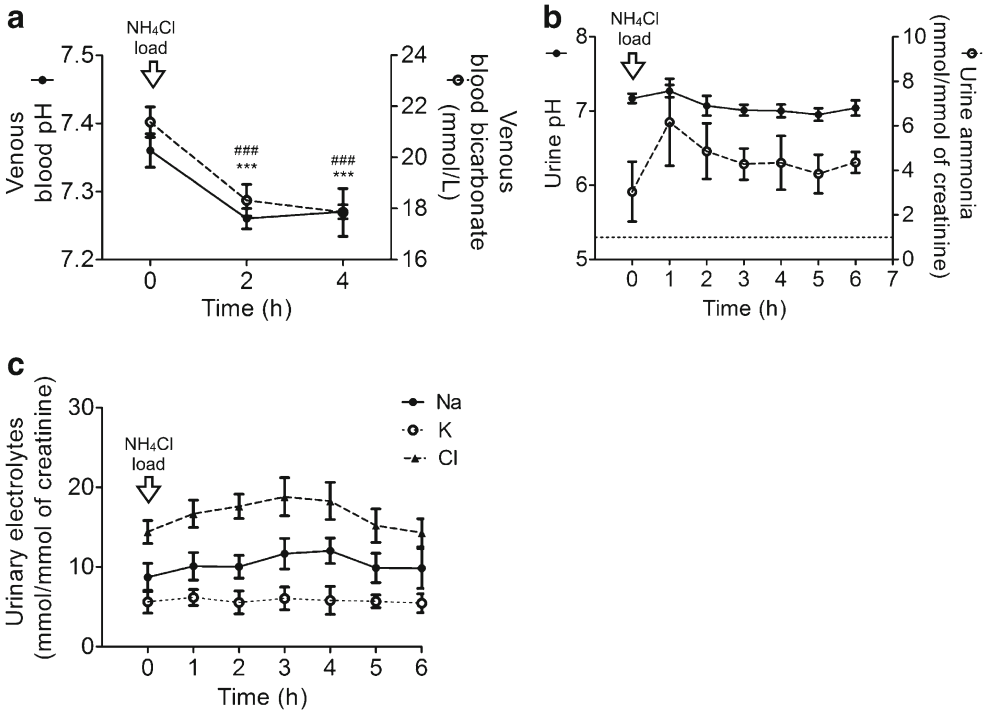

Fig. 9 Blood and urinary parameters during NH_4Cl loading in patients with inherited dRTA. Time 0 represents baseline (prior to NH_4Cl ingestion). Arithmetic means ± SEM of different parameters ($n = 5$). **a** Venous blood pH and bicarbonate. Asterisk and number sign indicate comparison of pH and bicarbonate, respectively with reference to baseline. **b** Urine pH and ammonia excretions indicate comparison of pH and ammonia, respectively, with reference to baseline. **c** Urinary Na, K, and Cl excretions indicate comparison of Na and Cl excretions, respectively, with reference to baseline. Urinary K excretion was not found significantly different during the course of experiment. Data are means ± SEM; $n = 5$. $^{*,\#}P < 0.05$, $^{**,\#\#}P < 0.01$, $^{***,\#\#\#}P < 0.001$ compared to baseline

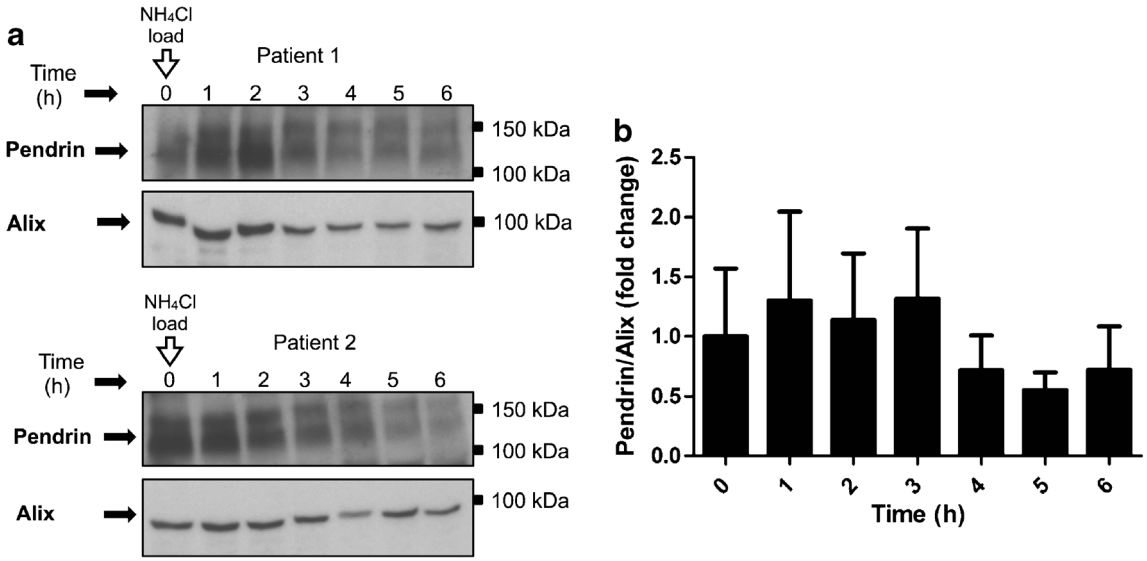

Fig. 10 Effect of NH_4Cl loading on pendrin abundance in urinary exosomes of patients with inherited dRTA. **a** Representative immunoblots of urinary exosomes isolated from two participants, probed with pendrin (upper panel) or alix (lower panel) antibodies. **b** Quantification of immunoblots of five participants. All data were normalized to the respective baseline. Data are means ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to baseline

base conditions. Investigation of pendrin protein in human subjects is not possible with conventional experimental setups. Therefore, we chose to study regulation of pendrin in humans by employing urinary exosomes, which contain apical membrane proteins of nephron-lining epithelial cells [17, 19].

The acute pendrin regulation we report here may be part of a complex adaptation mechanism by kidneys to different acid, base, and extracellular volume changes. Pendrin KO mice fail to secrete bicarbonate when subjected to alkali loading, indicating the importance of pendrin in adaptation of the mouse CCD to an alkali load [25]. Microperfused CCDs isolated from acidotic rats that underwent 4 days of NH_4Cl loading demonstrated a significant reduction in apical Cl^-/HCO_3^- exchanger activity and pendrin mRNA and protein [18]. In another study, 7 days of NH_4Cl treatment in rats led to similar findings while $NaHCO_3$ treatment caused a significant increase in pendrin expression [8]. Even after a short 1-day acid load, pendrin protein expression was reduced and pendrin was shifted from apical membranes to a more cytosolic localization along with reduction in pendrin positive cells. In contrast, after a 1-day alkali load, pendrin was found predominantly at the apical membrane [42]. These findings clearly indicate the role of pendrin in the adaptive response to acid or alkali loading, which is predominantly at the posttranslational level.

Our results on the acute and dramatic pendrin downregulation following an acute NaCl load deserve a special comment. Pendrin in the rodent kidney is regulated in response to alterations in chloride balance [22, 37]. Chronic administration of DOCP increases pendrin mRNA expression and cell surface abundance of pendrin in intercalated cells and pendrin KO mice are resistant to mineralocorticoid-induced hypertension [40]. Pendrin has been proposed to act in concert with the Na^+-dependent Cl^-/HCO_3^- exchanger NDBCE to mediate electroneutral NaCl reabsorption in the CNT and CCD [15]. The mechanisms of how changes in chloride intake modulate pendrin expression and localization remain unknown, but some evidence suggests that luminal chloride may play an important role [22]. Thus, the downregulation of pendrin abundance in urinary exosomes observed upon NaCl administration may involve, at least in part, chloride-dependent pathways. But regardless of the mechanism involved, our results obtained in humans are in line with rodent data and strongly support the involvement of pendrin in the electroneutral NaCl absorption in the distal nephron of the mammalian kidney, as previously proposed [15].

The salient finding of the present study is the dramatic downregulation of pendrin levels within 3–4 h following acid ingestion and upregulation after 1 h of alkali loading. These results indicate that pendrin is differentially regulated by acid and alkali challenges. We believe that this is the first demonstration of such a rapid process of adaptation to acidosis and alkalosis by pendrin in the human kidney. This kind of functional rapid adaptive response to acidosis has been recorded earlier ex-vivo in rabbit CCD tubules. Normally, CCDs taken from control rabbits secret net HCO_3^-, but after 3 h of exposure to low pH in vitro, the polarity of HCO_3^- flux reverses to that of net HCO_3^- absorption [27]. The polarity of net HCO_3^- transport is shifted from secretion to absorption after rapid in vitro metabolic acidosis. In another study, CCDs incubated at pH 6.8 reversed HCO_3^- flux from net secretion to absorption, whereas incubation for 3 h at pH 7.4 did not [36].

Based on our data of acute pendrin regulation, we speculate that the exosomal up- and downregulation of pendrin after alkali or acid loading is a reflection of adaptive changes occurring in CNT and CCD. Purkerson et al. have shown changes of subcellular pendrin localization after acid and alkali loading, indicating that the regulation of pendrin in type B intercalated cells occurs, at least in part, via shuttling between apical membrane and subapical compartments [20]. In analogy, we speculate here that such shuttling also occurs in the human kidney and results in alterations of pendrin protein abundance in urinary exosomes. In support of our hypothesis, such subapical to apical trafficking and concomitant alterations in the exosomal content has been described for several other apical membrane proteins, including the sodium/chloride co-transporter (NCC) [39] or aquaporin 2 (AQP2) [12, 35]. Clearly, additional studies comparing exosomal pendrin abundance with histological quantification of pendrin expression in renal biopsies or nephrectomy preparations are needed to substantiate this claim.

In this report, we also highlighted the differential regulation of pendrin levels in inherited dRTA patients. Along with lower baseline pendrin levels, the NH_4Cl-induced pendrin downregulation was blunted in dRTA patients compared to healthy subjects. Previous case reports suggested that patients with dRTA have low or undetectable levels of renal pendrin expression [14, 38]. Reduced pendrin levels may be an important contributor to the inappropriate loss of NaCl observed in dRTA patients [10]. DRTA patients recruited in our study had mutations in V-ATPase subunits (Table 1). This defect causes an impairment in tubular H^+ secretion with alkaline urine pH and development of systemic acidosis [13, 34, 46]. High luminal pH or intracellular acidosis is likely mediators of the low exosomal pendrin abundance observed in dRTA patients. However, the V-ATPase is also expressed basolaterally in type B intercalated cells; we therefore cannot exclude a direct effect of the mutations on type B intercalated cells. Unfortunately, we did not have patients with AE1 mutations available for study—the latter would be a type A intercalated cell-specific transporter and allow further mechanistic investigation. Furthermore, Eladari and co-workers demonstrated paracrine (PGE2, ATP) crosstalk between type A and B intercalated cells and a disruption of this signaling axis in ATP6V1B1 mutant mice [10]. Thus, alterations in paracrine signaling may be another reason for altered exosome composition of B type intercalated cells in patients with V-ATPase subunit mutations.

In summary, pendrin can be detected and quantitatively assessed in human urinary exosomes by immunoblotting. Our results suggest that pendrin abundance in urinary exosomes is altered within a few hours by acute acid, alkali, or salt loading, probably via mechanisms involving translocation of this apical protein. In contrast, pendrin abundance in urinary exosomes is greatly reduced in patients with inherited dRTA and not altered upon oral NH_4Cl loading.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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