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Exceptional Case

Loss of kAE1 expression in collecting ducts of end-stage kidneys from a family with SLC4A1 G609R-associated distal renal tubular acidosis

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Abstract

Distal renal tubular acidosis caused by missense mutations in kidney isoform of anion exchanger 1 (kAE1/SLC4A1), the basolateral membrane Cl−/HCO3− exchanger of renal alpha-intercalated cells, has been extensively investigated in heterologous expression systems but rarely in human kidneys. The preferential apical localization of distal renal tubular acidosis (dRTA)-associated kAE1 mutants R901X, G609R and M909T in cultured epithelial monolayers has not been examined in human kidney. Here, we present kidney tissues from dRTA-affected siblings heterozygous for kAE1 G609R, characterized by predominant absence rather than mistargeting of kAE1 in intercalated cells. Thus, studies of heterologous recombinant expression of mutant proteins should be, whenever possible, interpreted in comparison to affected patient tissues.

Key words: distal renal tubular acidosis, genetics, kAE1, kidney isoform of anion exchanger 1, slc4a1

Background

Familial distal renal tubular acidosis (dRTA) is caused by impaired renal acid-base homeostasis and can be accompanied by nephrocalcinosis, nephrolithiasis, hyperchloremia, hypokalemia and polyuria. If not diagnosed and treated early with bicarbonate replacement, patients display elevated risks of pyelonephritis and progression to end-stage renal disease (ESRD). The underlying defects in collecting duct acid secretion by alpha-intercalated cells (α-IC) reflect recessive loss-of-function mutations of ATP6B1 (vacuolar H+-ATPase β1 subunit) [1], ATP6VOA4 (vacuolar H+-ATPase α4 subunit) [2, 3] and CA2 (carbonic anhydrase II) [4, 5], as well as dominant and recessive loss-of-function mutations of SLC4A1 [kidney isofrom of anion exchanger 1 (kAE1)] [6–12]. Although heterologous expression of these mutant gene products has allowed proposal of disease mechanisms, the pathogenic mechanisms of these mutant proteins in dRTA patient kidneys remain unclear.

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kAE1 mediates electroneutral Cl−/HCO₃⁻ exchange across the basolateral membrane of α-IC. Localization of human kAE1 polypeptide expressed in polarized Madin-Darby canine kidney (MDCK) cell monolayers is also basolateral. However, dRTA-associated kAE1 mutants exhibit either retention in endoplasmic reticulum or Golgi (mutants R598H, S613F, C479W) or surface expression in nonpolarized or exclusively apical patterns (R901X, G609R, M909T) [12–17]. In contrast, the few reported histological studies of familial dRTA patient kidneys report partial or complete absence of kAE1 without evidence for apical membrane accumulation [18–22].

We report here the near-absence of detectable kAE1 in intercalated cells of ESRD nephrectomy specimens from siblings with dRTA secondary to the autosomal dominant mutation G609R in the kAE1 product of the SLC4A1 gene, in contrast to preferential apical localization of kAE1 G609R previously demonstrated in MDCK cell monolayers.

Case report

The 38-year-old male proband (III:1) was referred for recurrent, symptomatic nephrolithiasis diagnosed 10 years previously and was managed by lithotripsy and cysto-ureteroscopic extraction of calculi. Most stones contained 90% calcium phosphate/10% calcium oxalate. A few contained carbonate–apatite. Serum [HCO₃⁻] was 20–23 mM, serum calcium 9.1 mg/dL, blood urea nitrogen 12 mg/dL, serum creatinine 0.9 mg/dL and hematological indices were normal. A 24-h urine (3.85 L containing 1949 mg creatinine) collected while on citrate therapy (25 mEq/day) was remarkable hypocitraturia, with for values of pH 7.06, calcium 430 mg (normal <250), oxalate 41 mg (normal <40), citrate 58 mg (normal >450) and urate 0.67 g (normal <0.8). A second, consecutive day collection showed similar results.

Teenage onset of nephrolithiasis in the proband’s 74-year-old father (II:2) and in half-sisters III.3 (age 51 years) and III:4 (deceased at age 33 years from substance abuse) led to ESRD and transplant, without further stone disease. Autopsy of the 40-year-old paternal grandfather (I:1) revealed previously undiagnosed nephrocalcinosis. Half-brother III:2 was clinically unaffected. This personal and family history led to diagnosis of autosomal dominant dRTA in proband III:1, who has remained asymptomatic for 3 years on 15 mEq potassium citrate twice daily.

Genetic screening detected the heterozygous G609R variant of SLC4A1 (reported previously [12]) in the proband (III:1), his affected father (II:2) and surviving half-sister (III:3), whereas his unaffected half-brother (III:2) and mother expressed only wild-type alleles (Figure 1B). The cosegregating mutation was validated in genomic DNA of other family members by sequencing across SLC4A1 exon 15, as previously described [23]. Written consent was obtained under protocols approved by the Clinical Investigation Committees of Yale University School of Medicine and Beth Israel Deaconess Medical Center.

Fig. 1. Pedigree of dRTA family. (A) The heterozygous kAE1/SLC4A1 mutation G609R was detected in affected family members (filled symbols), but not in unaffecteds (open symbols). SLC4A1 genotype status is listed under symbols. (B) The heterozygous SLC4A1 mutation c.1825G>A encoding the SLC4A1/AE1 missense substitution G609R was detected in the proband (III:1) by DNA sequencing of both strands of PCR-amplified cDNA prepared from total RNA isolated from whole blood, as previously described [23]. The cosegregating mutation was validated in genomic DNA of other family members by sequencing across SLC4A1 exon 15, as previously described [24]. Written consent was obtained under protocols approved by the Clinical Investigation Committees of Yale University School of Medicine and Beth Israel Deaconess Medical Center.
reactivity by peroxidase-coupled antibody in control and patient tissue. Paraffin blocks from affected individuals III:3 and III:4 revealed grossly apparent regions of calcification. Sections from blocks with minimal calcification revealed chronic fibrotic and inflammatory changes consistent with the clinical diagnoses of chronic pyelonephritis and nephrocalcinosis. Tubules were separated by enlarged fibrotic interstitial spaces containing congested vessels. kAE1 immunostaining in tubular cells appeared absent, although eAE1 immunostaining was evident in red blood cells (arrows) of peritubular vessels and glomeruli. In some tubules, flattened epithelial cells appeared compressed by luminal debris and casts. In other tubules, granular, heterogeneous immunostaining throughout the cells suggested antigen aggregation or precipitation. The less intense, diffusely intracellular tubular immunostain, widely but not uniformly distributed among proximal tubular and other structures (Figure 2C and D), was consistent with low-level expression of the immunologically cross-reactive, more widely expressed AE2 anion exchanger, or with nonspecific immunostaining.

In fewer than 5% of visual fields, cells expressing abundant kAE1 were evident (Figure 3A and C, asterisks). These cells (Figure 3B and D, asterisks) were a minority among the many intercalated cells detectable in near-consecutive sections by immunostaining of vH+-ATPase (Figure 3B and D; compare with control in Figure 3E). Extremely rare tubular cells with modest apical or apicobasal enhancement of kAE1 staining (Figure 3A, arrows) were also noted.

Discussion

The heterozygous kAE1 G609R mutation was previously reported in one large kindred with autosomal dominant dRTA characterized by nephrocalcinosis, nephrolithiasis and progression to ESRD [12]. The recombinant kAE1 G609R polypeptide carrying an N-terminal HA-tag was stably expressed in polarized MDCK epithelial cell monolayers, where it accumulated predominantly at the cellular apical membrane. Other dominant dRTA-associated kAE1 missense mutant polypeptides accumulating predominantly in apical membranes of MDCK cells include the N-terminally HA-tagged variant 901X [18] and the N-terminally EGFP-tagged M909T [17], studies that defined a class I PDZ-binding motif at the kAE1 C-terminus. However, subcellular localization of these mutants in affected human kidney remains unknown.
Immunohistochemical study of kidney biopsies from 11 molecularly undiagnosed, primary dRTA patients pre-end-stage revealed absence of AE1 in seven specimens, reduction of AE1 in three and normal abundance in one [19]. In one patient with autoimmune polyendocrinopathy and dRTA of unknown etiology, kAE1 immunostaining was absent on kidney biopsy [32]. Additional reports have documented absence in kidney of immunoreactive vH^+^-ATPase in idiopathic dRTA [20] and absent renal immunoreactivity to vH^-ATPase and AE1 in Sjögren syndrome-associated dRTA [21, 22]. Human kidney kAE1 immunolocalization has been previously reported for two dRTA patients with known causative mutations. The resected end-stage kidney of a dRTA patient heterozygous for kAE1 R589H showed lack of immunospecific staining for AE1 and apparent reduction in intercalated cell number [28]. A kidney biopsy from a dRTA patient heterozygous for kAE1 S613F revealed reduced intercalated cell numbers with either intracellular or absent kAE1 immunostaining [22]. The preserved erythrocyte immunostaining for eAE1 in sections of kidneys expressing kAE1 mutants R589H, S613F and G609R reflects the erythrocyte’s increased tolerance for nominal AE1 haploinsufficiency compared with that of α-IC cells [7, 22, 28], perhaps explained in part by erythroid-specific expression of the chaperone protein, glycophorin A [33].

We have presented kAE1 immunostaining in resected kidney specimens of two siblings from a family with dRTA cosegregating with the heterozygous dRTA mutation SLC4A1 G609R. Intercalated cells were present in these end-stage kidney sections characterized by interstitial fibrosis and cellular atrophy, but kAE1-expressing intercalated cells were severely reduced in number and with reduced levels of expression. Only very rare cells exhibited potentially apical or apicobasal enhancement of kAE1-immunoreactive staining in the dRTA kidney tissue blocks available to us.

The finding of FSGS in the resected kidneys of both half-sisters III:3 and III:4 is of interest, in view of the identification of SLC4A1 as a protein-binding partner of nephrin [34]. Indeed, immunoreactive AE1 has been detected in mouse podocytes in culture and in mouse kidney glomeruli, and Ae1^−/− mice exhibited glomerulomegaly, glomerular basement membrane.

Fig. 3. Immunoperoxidase localization of kAE1 and vH-ATPase in kidney sections from dRTA patient III:3. (A and C) Non-representative regions of tissue in which AE1 staining (diaminobenzidine substrate, Vector Laboratories) is evident in diffuse distribution in occasionally encountered intercalated cells (asterisks). Less frequently encountered are candidate intercalated cells with basal-apical or apical enhancement of kAE1 staining (arrows in A). Red blood cells are evident in interstitial spaces. (B and D) Near-consecutive sections showing intercalated cells with diffuse vH^-ATPase staining (Novolink substrate, Vector Laboratories) with occasional apical enhancement. Asterisks indicate kAE1-positive cells in (A) and (B). Apical enhancement of vH^-ATPase staining is also evident in other collecting duct cells. (E) Unaffected ‘control’ kidney stained for vH^-ATPase. Scale bars: 20 µm (A-D) and 50 µm (E).
thickening, mesangial expansion and incompletely penetrant al-
buminuria [34]. The clinical presentation of dRTA can be
complex, as recently demonstrated by a novel autosomal recess-
itive dRTA associated with the homozygous SLC4A1 mutation
F524del initially diagnosed as a case of nephropnthesis [35].
Moreover, SLC4A1 mRNA abundance in isolated murine glom-
eruli as determined by SAGE may be as high as 8% of that in
isolated aldosterone-sensitive distal nephron [36], and mouse
glomerular SLC4A1 expression has been confirmed in sub-
sequent microarray studies [37, 38]. In contrast, RNaseq of micro-
dissected rat kidney revealed that SLC4A1 mRNA in isolated
glomeruli is only <0.1% of the level in isolated collecting ducts
[39]. Comparable human data remain unavailable. Immuno-
reactive kAE1 was not evident in non-erythroid glomerular cells
in our end-stage tissue samples.

The plasticity of polarized expression of heterologous trans-
gene products was recently highlighted by demonstration of cell-
line-specific differences in polarized membrane protein sort-
ing, attributable to differential expression of mu-adaptins [40].
Such cell-line-specific differences in protein trafficking pro-
teomes may explain classical differences in polarized expression
among independent clones of the same cell type [41]. Similar dif-
fences in SLC4A1 dRTA mutant polypeptide targeting were re-
cently observed in polarized cell lines and in mice. The dominant
dRTA mutant SLC4A1 R589H retained in the endoplasmic reticu-
ulum of polarized MDCK monolayers is localized normally at the
basolateral membrane in two mouse kidney epithelial cell
lines. Moreover, the corresponding mouse AE1 mutation R607H
also localizes to basolateral membranes of α-IC cells in intact kid-
ney, but with reduced expression levels in reduced numbers of
immunologically identifiable α-IC cells (Hennings JC, Eladari, D,
Huebner C, et al., manuscript in revision). Thus, mutant mem-
brane transporter targeting phenotypes can reflect genetic back-
grounds of host cells or organisms.

Our data are consistent with reduction or loss of kAE1 poly-
peptide expression as a dRTA mechanism in patients heterozy-
gous for kAE1 missense mutation G609R. With the rare possible
exceptions noted (Figure 3A), our data do not support apical mis-
targeting of mutant polypeptide as a pathogenic mechanism in
human kidney. However, establishment of a definitive human
kidney targeting phenotype of dRTA mutant kAE1 G609R must
await availability of morphologically intact kidney tissue from
dRTA patients of defined genotype.

Authors’ contribution
A.A.V. wrote the manuscript, recruited patients and collected tis-
tues. B.E.S. carried out genotyping and reviewed the manuscript.
Z.K.Z. performed immunohistochemistry and reviewed the manus-
cript. N.D.A. provided paraffin-embedded fixed tissues and
reviewed the manuscript. N.K.D. wrote the manuscript and
recruited patients. S.L.A. wrote the manuscript, carried out gen-
typing and performed immunohistochemistry.

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Conflict of interest statement
The results presented in this paper have not been published previ-
ously.

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