A Novel Missense Mutation in AE1 Causing Autosomal Dominant Distal Renal Tubular Acidosis Retains Normal Transport Function but Is Mistargeted in Polarized Epithelial Cells*

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Mutations in SLC4A1, encoding the chloride-bicarbonate exchanger AE1, cause distal renal tubular acidosis (dRTA), a disease of defective urinary acidification by the distal nephron. In this study we report a novel missense mutation, G609R, causing dominant dRTA in affected members of a large Caucasian pedigree who all exhibited metabolic acidosis with alkaline urine, prominent nephrocalcinosis, and progressive renal impairment. To investigate the potential disease mechanism, the consequent effects of this mutation were determined. We first assessed anion transport function of G609R by expression in Xenopus oocytes. Western blotting and immunofluorescence demonstrated that the mutant protein was expressed at the oocyte cell surface. Measuring chloride and bicarbonate fluxes revealed normal 4,4'-disothiocyanostilbene-2,2'-disulfonic acid-inhibitable anion exchange, suggesting that loss-of-function of kAE1 cannot explain the severe disease phenotype in this kindred. We next expressed epitope-tagged wild-type or mutant kAE1 in Madin-Darby canine kidney cells. In monolayers grown to polarity, mutant kAE1 was detected subapically and at the apical membrane, as well as at the basolateral membrane, in contrast to the normal basolateral appearance of wild-type kAE1. These findings suggest that the seventh transmembrane domain that contains Gly-609 plays an important role in targeting kAE1 to the correct cell surface compartment. They confirm that dominant dRTA is associated with non-polarized trafficking of the protein, with no significant effect on anion transport function in vitro, which remains an unusual mechanism of human disease.

Distal renal tubular acidosis (dRTA)† is a disease of defective urinary acidification characterized by impaired H+ secretion into the urine leading to metabolic acidosis. This disorder is usually accompanied by hypokalemia, metabolic bone disease, nephrocalcinosis, and/or nephrolithiasis (1–3). Mutations in SLC4A1 (MIM 109270), encoding the polytopic chloride-bicarbonate exchanger known as AE1, have been reported as the sole genetic cause of autosomal dominant dRTA (ddRTA) (MIM #179800) (4–9) and also rarely cause autosomal recessive dRTA (10–14). Although several different SLC4A1 mutations have been found to be involved in this disease, the molecular mechanisms of pathogenesis for these mutations are not completely defined.

In humans, the two main sites of AE1 expression are in erythrocyte cell membranes and, in the kidney, at the basolateral surface of α-intercalated cells in the collecting duct segment of the nephron. The encoded proteins are both products of SLC4A1, but because different promoters initiate transcription, they differ at their N termini, the renal isoform (kAE1) missing the first 65 amino acids found in the erythrocyte protein.

Reported SLC4A1 mutations associated with ddRTA include missense alterations of Arg-589 (R589H, R589S, and R589C) (4–8) and S613F (4); A>R901X (5). These mutations have been studied for their anion transport function and protein trafficking by expression in Xenopus oocytes and cultured mammalian cells, for example human embryonic kidney (HEK) and Madin-Darby canine kidney (MDCK) cells. When mutant R589H, R589C, or S613F AE1 proteins were expressed in Xenopus oocytes, they reached the cell surface and exhibited at worst only mildly reduced anion exchange activity (4, 6). Impaired trafficking of mutant R589H, R589C, and R589S kAE1 proteins was demonstrated when expressed in HEK 293 cells, and co-expression of wild-type kAE1 with these mutant proteins also revealed a dominant negative effect (15). Furthermore, it has been suggested that the R901X mutant retained normal anion exchange activity in Xenopus oocytes but failed to express at the cell surface of unpolarized MDCK cells (16) and HEK 293 cells (17). From all of these data, it has been concluded that mutant AE1 proteins maintain sufficient anion transport function but are defective in targeting to the cell surface. These results suggest that the explanation for AE1 mutations and ddRTA is not simple loss-of-function.

MDCK, Madin-Darby canine kidney; HA, hemagglutinin; PBS, phosphate-buffered saline.

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‡‡ The abbreviations used are: dRTA, distal renal tubular acidosis; ddRTA, autosomal dominant dRTA; HEK, human embryonic kidney; H11001, H11541 -diisothiocyanostilbene-2,2'-disulfonic acid.

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As kAE1 is specifically located in the basolateral membrane of Ω-intercalated cells in the distal nephron, an alternative reasonable hypothesis is that kAE1 mutations do not cause complete intracellular retention but instead result in mistargeting to the apical rather than basolateral membrane of these cells (13, 18). However, almost all the protein-targeting studies that demonstrated impaired trafficking of mutant kAE1 were conducted in non-polarized cells, so it is possible that these proteins may behave differently in Ω-intercalated cells. In support of this, we have recently demonstrated that kAE1-R901X is aberrantly targeted to the apical surface of polarized MDCK cells, which emphasizes the importance of polarized cell models for targeting studies of the membrane proteins (19).

In this study, we identified a novel missense mutation of the AE1 gene in a Caucasian family with dRTA. This mutation was further examined for its transport function in Xenopus oocytes and its trafficking in polarized MDCK cells.

**EXPERIMENTAL PROCEDURES**

**Subjects**—Several members of a Caucasian kindred with autosomal dominant dRTA were studied (Fig. 1A). All affected individuals were determined to have metabolic acidosis with alkaline urine, nephrocalcinosis, and progressive renal impairment. Control subjects consisted of 30 unrelated, unaffected individuals from the same ethnic background.

**Mutation Screening of SLCA41**—Each coding exon of the kidney isoform of SLCA41 (kAE1), including its flanking exon-intron boundaries, was individually amplified from genomic DNA of study patients. These PCR products were sequenced using ABI Prism® BigDye® terminator cycle sequencing (Applied Biosystems) on an ABI377 instrument. Identified sequence alterations were confirmed in family members and assessed in 30 controls by allele-specific PCR (20, 21) using primers BD3x15R-ASA-A (5′-GTC GGG TCA TCG AGG ACT ACA-3′) or BD3x15R-ASA-A (5′-GTC GGG TCA TCG AGG ACT ACA-3′) and BD3x15F-5′-CAG GGA GGT GGT AGA GAT AGT C-3′. A cDNA clone containing full-length kAE1 constructed in pHM6 was used as template for PCR amplification using the Expand® long template PCR system (Roche Applied Science) and specific primers to introduce a HindIII site, a Kozak consensus sequence, start codon, and hemagglutinin (HA) tag upstream of the second codon of kAE1, and a HindIII site downstream of the stop codon of the gene. This 2.5-kb PCR product was digested with HindIII and cloned into a vector containing the 5′- and 3′-untranslated regions of Xenopus β-globin (gift of Cecilia Canessa, Yale University) for expression in Xenopus oocytes. This wild-type kAE1 clone, pXG-kAE1, was used as a template for site-directed mutagenesis (QuikChange™ site-directed mutagenesis kit, Stratagene) to make a mutant clone, pXG-kAE1G609R. The sequence of these two cDNA constructs were verified by direct sequencing. pXG-kAE1 and pXG-kAE1G609R were linearized with NheI before transcription of cRNA using T7 RNA polymerase with the mMESSAGE mMICHEM™ kit (Ambion, Inc.), according to the manufacturer’s protocol.

**Chloride and Bicarbonate Transport in Xenopus Oocytes Expressing kAE1 Constructs**—Fresly harvested and manually dejellified Xenopus laevis oocytes were microinjected with 5 ng of cRNA or an equivalent volume of water and were maintained in ND66 medium (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.6) at 19 °C for 3–4 days. Chloride ion uptake was measured according to the method of Garcia and Lodish (22). Briefly, groups of 8–12 injected oocytes were incubated in ND66 containing 50 μM 36Cl⁻ (Amer sham Biosciences) at 19 °C for 1 h. Parallel groups of oocytes were pretreated with 100 μM DIDS in ND96 at 19 °C for 1 h, before addition of the 36Cl⁻. At the end of the incubation period with 36Cl⁻, oocytes were washed in a chloride-free medium (96 mM sodium gluconate, 2 mM potassium gluconate, 1 mM MgSO₄, 10 mM NaHCO₃, 1.8 mM CaCl₂, 10 mM NaHCO₃). This medium was removed and replaced with 250 μl of ND96/HCO₃⁻ solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 10 mM NaHCO₃). 100-μl samples were taken at 0, 10, 20, 30, and 60 min after the addition of 36Cl⁻, and the pH of the medium was read using a micro pH electrode (Hanna HI 1083, Jencons/PLS). The HCO₃⁻ flux/oocyte is given by the equation

\[ J = \beta_0 \cdot \Delta pH \cdot v_{o} \cdot 10^{-2} \]  

where \( J \) is the buffer capacity (23).

**Membrane Preparation and Western Blot Analysis**—Total oocyte membranes were prepared according to previous methods (24–26). Groups of 20 water- or cRNA-injected oocytes were homogenized using a tissue-coring device and sonicated three times. Control subjects were blocked for 1 h in 5% skim milk in PBST (PBS with 0.05% Tween 20) and then incubated with a rat monoclonal α-HA antibody at 1:200 dilution in PBST containing 0.5% skim milk for 1 h. After three 10-min washes in PBST, membranes were incubated for 1 h with 1:5,000 horseradish peroxidase-conjugated rabbit α-rat Ig. After three 10-min washes in PBST, specific proteins were detected using the Lumiglo chemiluminescent substrate kit (Insight Biotechnology) according to the manufacturer’s instructions.

**Oocyte Immunofluorescence**—Three days after injection with water, wild-type, or mutant kAE1 cRNA, Xenopus oocytes were fixed for 4 h in methanol at −20 °C. The fixative was replaced with 30% (v/v) sucrose in PBS (pH 7.4) at 4 °C overnight for cryopreservation. Oocytes were then incubated in OCT compound (OCT and DMSO, in 1:10 ratio) and frozen on a dry-ice slide. Cryosections were cut on a cryostat and mounted on electrostatically charged microscope slides (Superfrost Plus, BDH). These cryosections were preincubated with PBS, 1% bovine serum albumin at room temperature for 15 min and incubated with primary antibody (mouse monoclonal anti-HA antibody) at 4 °C overnight. Primary antibody was omitted in control experiments. After three 10-min washes in PBS, sections were incubated with fluorescein isothiocyanate-labeled anti-mouse Ig antibody (1:200 dilution) at room temperature in the dark for 1 h. Following three further washes in PBS, sections were mounted using Vectashield mounting medium (Vector Laboratories). Immunofluorescence was visualized on a Nikon ECLIPSE E600 microscope equipped with the Nikon Filter G1 for Expression in Xenopus Oocytes—a cDNA construct containing full-length kAE1. Immunofluorescence staining of the 5′- and 3′-untranslated regions of Xenopus β-globin (gift of Cecilia Canessa, Yale University) for expression in Xenopus oocytes. This wild-type kAE1 clone, pXG-kAE1, was used as a template for site-directed mutagenesis (QuikChange™ site-directed mutagenesis kit, Stratagene) to make a mutant clone, pXG-kAE1G609R. The sequence of these two cDNA constructs were verified by direct sequencing. pXG-kAE1 and pXG-kAE1G609R were linearized with NheI before transcription of cRNA using T7 RNA polymerase with the mMESSAGE mMICHEM™ kit (Ambion, Inc.), according to the manufacturer’s protocol.

**Cell Culture and Transfection**—MDCK cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum; 1% penicillin, streptomycin, and 1% glutamine (Sigma) and maintained in ND66 containing 5% CO₂ at 37 °C with 5% CO₂ in 95% air. Cell cultures were transfected with 3 μg of pcDNA3.1-kAE1 or pcDNA3.1-KAE1G609R using FuGENE 6 transfection reagent (Roche Diagnostics). Membrane proteins were then pelleted at 100,000 × g for 30 min to give a total membrane fraction. Pellets were resuspended in 20 μl of homogenization buffer, and protein concentration was determined by Bradford protein assay (Bio-Rad). Protein samples were denatured at 95 °C for 10 min, subjected to electrophoresis on a 7.5% SDS-polyacrylamide gel (loading 40 μg/lane), and transferred to nitrocellulose membranes (Schleicher and Schuell). Membrane proteins were blocked for 1 h in 5% skim milk in PBST (PBS with 0.05% Tween 20) and then incubated with a rat monoclonal α-HA antibody at 1:200 dilution in PBST containing 0.5% skim milk for 1 h. After three 10-min washes in PBST, membranes were incubated for 1 h with 15,000 horseradish peroxidase-conjugated rabbit α-rat Ig. After three 10-min washes in PBST, specific proteins were detected using the Lumiglo chemiluminescent substrate kit (Insight Biotechnology) according to the manufacturer’s instructions.

**Immunofluorescence**—Three days after injection with water, wild-type, or mutant kAE1 cRNA, Xenopus oocytes were fixed for 4 h in methanol at −20 °C. The fixative was replaced with 30% (v/v) sucrose in PBS (pH 7.4) at 4 °C overnight for cryopreservation. Oocytes were then incubated in OCT compound (OCT and DMSO, in 1:10 ratio) and frozen on a dry-ice slide. Cryosections were cut on a cryostat and mounted on electrostatically charged microscope slides (Superfrost Plus, BDH). These cryosections were preincubated with PBS, 1% bovine serum albumin at room temperature for 15 min and incubated with primary antibody (mouse monoclonal anti-HA antibody) at 4 °C overnight. Primary antibody was omitted in control experiments. After three 10-min washes in PBS, sections were incubated with fluorescein isothiocyanate-labeled anti-mouse Ig antibody (1:200 dilution) at room temperature in the dark for 1 h. Following three further washes in PBS, sections were mounted using Vectashield mounting medium (Vector Laboratories). Immunofluorescence was visualized on a Nikon ECLIPSE E600 microscope equipped with the Nikon Filter G1 for Expression in Xenopus Oocytes—a cDNA construct containing full-length kAE1. Immunofluorescence staining of the 5′- and 3′-untranslated regions of Xenopus β-globin (gift of Cecilia Canessa, Yale University) for expression in Xenopus oocytes. This wild-type kAE1 clone, pXG-kAE1, was used as a template for site-directed mutagenesis (QuikChange™ site-directed mutagenesis kit, Stratagene) to make a mutant clone, pXG-kAE1G609R. The sequence of these two cDNA constructs were verified by direct sequencing. pXG-kAE1 and pXG-kAE1G609R were linearized with NheI before transcription of cRNA using T7 RNA polymerase with the mMESSAGE mMICHEM™ kit (Ambion, Inc.), according to the manufacturer’s protocol.
followed by further washes in PBS. Nonspecific binding was blocked with 1% bovine serum albumin in PBS for 15–30 min, and filters were then incubated with primary antibody (1:100 dilution of rat monoclonal anti-HA (Roche Applied Science)) for 1 h. Washing twice with PBS was followed by incubation with half-strength blocking solution for 5 min. These steps were repeated three times. Filters were then incubated with secondary antibody (1:150 dilution of goat anti-rat Ig conjugated to Texas Red) and counter-stained with 1:200 dilution of peanut lectin conjugated to fluorescein isothiocyanate for 30 min in the dark. Washing with PBS and incubation with half-strength blocking solution steps were performed as before. After mounting with Antifade (Molecular Probes, Eugene, OR), the expression of protein was examined by visualization under a Zeiss LSM510 confocal microscope.

RESULTS

Kindred RTA-1—The index case (Fig. 1A, III:8) presented at the age of 6 with ureteric colic and was found to have a metabolic acidosis with concomitant alkaline urine and prominent nephrocalcinosis with calculi. In common with his mother and four of her siblings, her father, and two of her aunts, a diagnosis of renal tubular acidosis was made. Compliance with alkali therapy was poor, and he suffered recurrent problems with stones and progressive renal impairment, requiring dialysis from 1995.

In 1996, he received an allograft from his unaffected sister. His native kidneys were removed prior to transplantation. Histological examination revealed bilateral end-stage tubulointerstitial disease with heavily calcified renal parenchyma and multiple renal calculi. He has remained well since transplantation, excepting a reversible episode of acute cellular rejection 6 months post-engraftment.

Extending this kindred shows that several first cousins have also been diagnosed with RTA (Fig. 1A). Renal tract calcification is a prominent feature in this kindred, as is premature renal impairment. Cousin III:1 is awaiting a renal transplant at present.

Identification of kAE1 G609R Mutation—DNA sequence studies of SLC4A1 in the index case revealed a heterozygous substitution of G by A at nucleotide 1825, which results in the missense kAE1 mutation, glycine (GGG) to arginine (AGG) at a position 609 (G609R). Each DNA sample was analyzed for both wild-type allele, G, and mutant allele, A. The same PCR product (216 bp) representing alleles G and A was detected in affected individuals III:8, II:6, and III:3, whereas only that of allele G was detected in III:9 (unaffected). M, 100-bp DNA ladder. -ve, PCR amplification using water to replace DNA. D, approximate location of G609R in transmembrane domain 7. Amino acid sequences (28) between transmembrane domains 6 (TM6) and 7 (TM7) are shown.
Genomic DNA from each individual was amplified in two separate reactions, with either a pair of wild type-specific or mutant-specific primers, which generated the same PCR product size of 216 bp of G or A allele. The results showed that the mutation segregates with disease phenotype in available family members III:8, II:6, and III:3 (Fig. 1C). The mutation was present heterozygously in all three affected individuals but absent both from an unaffected family member (III:9) and from 30 control subjects.

Expression Studies of kAE1 in Xenopus Oocytes—To determine heterologous expression of the G609R kAE1 mutant, wild-type or mutant cRNAs encoding full-length human kAE1 incorporating an N-terminal HA tag were first expressed in Xenopus oocytes. Western blotting of total membrane fractions prepared from microinjected oocytes using an anti-HA antibody demonstrated a single major protein of molecular mass 95 kDa, corresponding to kAE1, in both wild-type and mutant cRNA-injected oocytes, which was absent in control (water-injected) oocytes (Fig. 2). Immunofluorescence was also performed to confirm the expression of wild-type and mutant kAE1 at the plasma membrane of the Xenopus oocyte. The immunolocalization of kAE1 in cryosectioned oocytes injected with wild-type or mutant kAE1 cRNA showed expression of the protein at the cell surface in both cases (Fig. 3), whereas this immunofluorescence was absent in oocytes injected with water or in negative controls where primary antibody was omitted. These results indicate that the mutant G609R protein maintained normal stability and was expressed at the cell surface of Xenopus oocytes in comparable amounts to wild-type kAE1.

Functional Studies of Mutant kAE1 in Xenopus Oocytes—To investigate whether the mutation affects kAE1 transport activity, chloride influx and bicarbonate efflux assays were performed. Comparison of 36Cl− uptake mediated by wild-type-, mutant-, or water-injected (control) oocytes assayed with or without addition of DIDS, a specific covalent inhibitor of AE1 (27), is shown in Fig. 4. These results demonstrate significant 36Cl− uptake following wild-type or mutant kAE1 cRNA injection compared with water injection but no significant difference between wild-type and mutant cRNA. Mean uptake of 36Cl− by wild-type and mutant kAE1 proteins was 13.36 ± 0.48 and 13.81 ± 0.51 nmol of Cl−/oocyte/h, respectively (p = 0.54). We also determined 36Cl− uptake by groups of oocytes injected with wild-type or mutant cRNA, which were pretreated with 100 μM DIDS for 1 h before the assay. The 36Cl− uptakes by both wild-type and mutant kAE1 proteins were significantly decreased by DIDS but were not significantly different from each other (4.42 ± 0.74 versus 4.20 ± 0.78 nmol of Cl−/oocyte/h; p = 0.85). Endogenous 36Cl− influx was 0.92 ± 0.25 (no DIDS) nmol of Cl−/oocyte/h and 0.81 ± 0.19 (preincubation with DIDS) nmol of Cl−/oocyte/h in a group of oocytes injected with water.

Since kAE1 functions as a one-to-one Cl−/HCO3− exchanger (28) across the basolateral membrane of α-intercalated cells, we wanted to confirm this function of the mutant protein by assaying HCO3− efflux. Although HCO3− efflux could not be measured from single oocytes, we developed a method to determine its efflux from batches of 30–40 injected oocytes. As expected, this revealed similar results to the 36Cl− influx study. The calculated HCO3− efflux induced by oocytes injected with wild-type or mutant kAE1 cRNA was 15.5 and 16.4 nmol of HCO3−/oocyte/h, respectively. Taken together, these experiments demonstrate that this kAE1 mutant protein retains normal anion (Cl−/HCO3−) transport activity.

Localization of kAE1 in Transfected MDCK Cells—MDCK I cells transfected with kAE1-G609R or kAE1 demonstrated markedly different expression patterns of these proteins (Fig. 5). As reported previously (19), wild-type kAE1 was observed exclusively at the basolateral surface of cells grown to polarity on Transwell filters (Fig. 5A). In contrast, the G609R mutant protein was visualized in a widespread distribution that included the basolateral surface and considerable subapical localization, together with some apical co-localization with the surface marker peanut lectin (Fig. 5B).
DISCUSSION

Initial efforts in this study were directed toward identifying the genetic cause of ddRTA in a large family who exhibited nephrocalcinosis and renal impairment and resulted in the isolation of a novel SLC4A1 mutation. This glycine at position 609 is conserved among all known vertebrate AE1 gene family sequences, providing an idea of functional importance of this residue. Gly-609 is predicted to lie within the seventh transmembrane domain of the polytopic AE1 protein, toward its cytoplasmic end (29). Gly-609 is also located close to two residues where missense mutations have been reported, in codons Arg-589 and Ser-613, reinforcing the importance of this region of the multiple transmembrane-spanning half of the molecule.

The mutation reported here results in substitution of a small polar uncharged amino acid by a positively charged side-chain residue. It is not clear what effect this might have on the local structure of this particular α-helix, but our studies demonstrate that it does not affect overall synthesis of the protein. It seems likely that if this glycine is normally situated within the lipid bilayer, then its replacement by arginine might disrupt nearby conformation and thus binding of another carrier or targeting molecule, but this must remain a matter for speculation at present.

In investigating the potential disease mechanism, functional consequences of this G609R kAE1 mutation on anion transport were assessed by a combination of Xenopus oocyte and polarized epithelial cell studies. The expression systems used for functional and trafficking studies of membrane proteins are important. Xenopus oocytes provide an ideal expression model for transport function of ion transporters both because their large size makes handling and manipulation relatively straightforward and because they do not express endogenous kAE1, permitting measurement of the functional activity of the pure mutant proteins (22, 30). However, oocytes cannot be used for examination of potential polarized sorting defects (31). In the absence of a definitive α-intercalated cell model, cultured mammalian cells such as MDCK cells, a canine collecting duct cell line that forms tight polarized monolayers, have proven suitable for protein-trafficking studies. In common with other plasma membrane-resident proteins, it remains difficult to transfect these cells with heterologous kAE1 cDNA, and they are not quite an ideal cell model for α-intercalated cells as they are derived originally from principal cells, the other major cell type in the collecting duct. Further studies to quantify the level of apical expression of mutant kAE1 would be advantageous if a stable line with sufficient expression could be generated, but we have to date been unable to achieve significant expression levels in stable lines to render this feasible.

In terms of overall mutation detection in this disease, R589H, R589C, and R589S were reported in five families, three families, and one family, respectively (4–8), whereas S613F was present in one kindred (4). The pathogenetic mechanisms of these previously reported mutations remain unclear, functional studies having concluded that anion exchange is at worst –8), whereas S613F was present in one kindred (4). The pathogenetic mechanisms of these previously reported mutations remain unclear, functional studies having concluded that anion exchange is at worst mildly diminished (4, 6). It has been suggested that intra-cellular retardation of R589H and R901X mutants expressed in non-polarized cells is the dominant negative effect explaining the lack of urine acidification and dominant nature of ddRTA (15, 17). However, kAE1-R901X expression in MDCK cells grown to polarity clearly results in aberrant surface expression with some mutant protein appearing at the apical surface (19) in common with the findings reported here; on the other hand, Arg-589 mutants have yet to be assessed in polarized cells, and observed results for the different mutations may be different because of this. In addition, variants of hereditary spherocytosis where AE1 haploinsufficiency occurs are not associated with a renal abnormality, suggesting that loss of 50% of the encoded protein is not sufficient to explain ddRTA (32). It is entirely possible that the mechanisms associated with different mutations may not be the same, but alternative explanations for these other cases have yet to emerge.

The spectrum of phenotypic severity in distal RTA is wide, ranging from the asymptomatic occurrence of stones in adulthood to childhood growth impairment and rickets. Affected members of this particular kindred exhibited a striking degree of nephrocalcinosis and premature renal failure. Although the number of reported cases is too small to permit definitive genotype-phenotype correlations, it is notable that they share this severity with the kindred carrying the R901X mutation where mistargeting of the mutant protein is also a feature. It is tempting to speculate that this is because these mutations result in net bicarbonate secretion into the urine rather than overall functional cellular failure, but this has not been assessed. One way to address this would be to measure urine pCO2, predicting that in affected members of this kindred and the R901X kindred, it would not be reduced to the extent usually observed in ddRTA. It is unfortunate that severe nephrocalcinosis and consequent anatomical disruption renders the interpretation of pCO2 levels unreliable.

Taken together, our finding of improper trafficking of this novel G609R mutation associated with ddRTA suggests that this conserved residue in the seventh transmembrane span plays an important role in correct protein targeting, without a deleterious effect on anion transport function. As AE1 is a functional dimer (33), we would expect that both homo- and heterodimers of wild-type and mutant protein could appear in the apical compartment. This effect of protein behavior of kAE1, observed previously with the C-terminal truncating mutation, leads to impaired urine acidification by disrupting the delicate electrochemical balance across α-intercalated cells resulting in severe disease. Mistargeting of mutant low density lipoprotein receptors in one form of inherited hyperlipidemia (34) is the best known other example of a mechanism of disease that remains very unusual, with few precedents.

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A Novel Missense Mutation in AE1 Causing Autosomal Dominant Distal Renal Tubular Acidosis Retains Normal Transport Function but Is Mistargeted in Polarized Epithelial Cells

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