

Novel AE1 Mutations in Recessive Distal Renal Tubular Acidosis

Loss-of-Function Is Rescued by Glycophorin A

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Abstract

The *AE1* gene encodes band 3 $\text{Cl}^-/\text{HCO}_3^-$ exchangers that are expressed both in the erythrocyte and in the acid-secreting, type A intercalated cells of the kidney. Kidney *AE1* contributes to urinary acidification by providing the major exit route for HCO_3^- across the basolateral membrane. Several *AE1* mutations cosegregate with dominantly transmitted nonsyndromic renal tubular acidosis (dRTA). However, the modest degree of in vitro hypofunction exhibited by these dRTA-associated mutations fails to explain the disease phenotype in light of the normal urinary acidification associated with the complete loss-of-function exhibited by *AE1* mutations linked to dominant spherocytosis. We report here novel *AE1* mutations linked to a recessive syndrome of dRTA and hemolytic anemia in which red cell anion transport is normal. Both affected individuals were triply homozygous for two benign mutations M31T and K56E and for the loss-of-function mutation, G701D. *AE1* G701D loss-of-function was accompanied by impaired trafficking to the *Xenopus* oocyte surface. Coexpression with *AE1* G701D of the erythroid *AE1* chaperonin, glycophorin A, rescued both *AE1*-mediated Cl^- transport and *AE1* surface expression in oocytes. The genetic and functional data both suggest that the homozygous *AE1* G701D mutation causes recessively transmitted dRTA in this kindred with apparently normal erythroid anion transport. (*J. Clin. Invest.* 1998. 102:2173–2179.) Key words: kidney • collecting duct • pH • red cell membrane • *Xenopus* oocyte • human genetics

Introduction

To maintain acid-base homeostasis, the kidney must reclaim all filtered bicarbonate and excrete additional acid sufficient in quantity to match that produced by systemic metabolism. Although the proximal tubule can reabsorb up to 80–90% of fil-

tered bicarbonate, the final stage of acid excretion occurs in the distal nephron, mediated largely by type A intercalated cells (IC)¹ of the cortical and outer medullary collecting ducts. Inherited defects in this urinary acidification process, known as primary distal renal tubular acidosis (dRTA), are manifest by inappropriately alkaline (less than maximally acidified) urine in the presence of systemic metabolic acidosis (complete dRTA) or in response to an imposed acid load (incomplete dRTA) (1). Primary dRTA is inherited in both autosomal dominant and recessive patterns and exhibits a spectrum of clinical severity. Patients frequently present with growth retardation, osteomalacia, nephrocalcinosis, and/or nephrolithiasis (2, 3). These latter conditions can predispose to pyelonephritis and eventual renal insufficiency. If detected in time, therapeutic correction of the acidosis by alkali administration leads in most cases to improvement of biochemical abnormalities and resumption of normal growth.

Recently, autosomal dominant dRTA has been found to be associated in several kindreds with mutations in the *AE1* gene (4–6). This gene encodes both the erythroid (eAE1) and the kidney (kAE1) isoforms of the band 3 protein (7). eAE1 contributes structurally to red cell integrity (8) and to organismic respiratory function by increasing blood CO_2 carrying capacity (7). kAE1 lacks the amino-terminal 65–amino acid residues of eAE1 (9, 10) and provides the major exit route for HCO_3^- in exchange for Cl^- across the basolateral membrane of the type A IC during urinary acidification (11).

The few *AE1* mutations presently defined in autosomal dominant dRTA include missense mutations in codon R589 (R589H, R589S, R589C) of transmembrane domain 6 (TM6) found in multiple unrelated families (4, 5), the S613F mutation in TM7 in one family (4), and an 11–amino acid deletion at the carboxy terminus (6) in one family. Functional analysis of the most extensively studied R589 mutation, however, reveals only modest reductions in *AE1*-mediated $^{36}\text{Cl}^-$ transport when expressed in *Xenopus* oocytes, and the S613 mutation is associated with upregulation of anion transport. Similarly mild phenotypes are also evident in *AE1*-mediated sulfate transport in patient red cells. Remarkably, these mild defects are less severe than those associated with *AE1* mutations of *AE1*-deficient autosomal dominant hereditary spherocytosis, a disease not known to be accompanied by classic dRTA (4, 5). Thus,

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Received for publication 13 October 1998.

J. Clin. Invest.

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0021-9738/98/12/2173/07 \$2.00

Volume 102, Number 12, December 1998, 2173–2179

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1. Abbreviations used in this paper: DIDS, 4,4'-diisothiocyanato-4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; dRTA, distal renal tubular acidosis; eAE1, erythroid *AE1*; GPA, glycophorin A; IC, intercalated cell; kAE1, kidney *AE1*; RT, reverse transcription; SSCP, single-strand conformational polymorphism; TM6, transmembrane domain 6.

despite the genetic linkage of dRTA to the AE1 locus and the definition of cosegregating mutations that alter encoded polypeptide sequence, the mechanisms by which mutant AE1 polypeptides cause dRTA remain unclear.

Here, we report a family in which recessively transmitted dRTA is associated with homozygosity for three mutations in the *AE1* gene, two of which are novel. AE1 expression and function were studied in red cells of affected and unaffected family members, and in *Xenopus* oocytes injected with mutant and wild-type cRNA. The data revealed a loss-of-function phenotype of the AE1 mutation that can be rescued by coexpression of the erythroid chaperonin protein, glycophorin A. The functional phenotype of the mutant AE1 polypeptide suffices to explain the clinical phenotype. These functional data are the first to agree with genetic evidence that mutations in the *AE1* gene can cause dRTA.

Methods

Patients. The male proband presented in Thailand at age 3.5 yr with a history of lethargy, anorexia, and slow growth. Physical exam noted height and weight < 3rd percentile, pallor, and hepatosplenomegaly. Hypokalemia, hyperchloremic metabolic acidosis, and normal creatinine were accompanied by isosthenuria and alkaline urinary pH, bilateral nephrocalcinosis, and rachitic bone changes. Mild anemia (Hct

11 g/dl) with microcytosis (MCV 65 fL), reticulocytosis (12%), and a peripheral smear consistent with a xerocytic type of hemolytic anemia (Fig. 1 C), were accompanied by homozygosity for hemoglobin E (Hb E/E), a clinically benign hemoglobinopathy frequently encountered in Southeast Asia (12).

3 yr later, the proband's younger sister was evaluated at age 1 yr for delayed growth and exhibited similar findings of hemolytic anemia (Fig. 1 D), nephrocalcinosis, rachitic bone changes, and hypokalemic metabolic acidosis. Both affected sibs failed maximally to concentrate urine after dehydration, were unresponsive to dDAVP, and failed to acidify urine (as determined by urinary pH, anion gaps, and NH_4^+ excretion). These two sibs were diagnosed with complete dRTA and responded to treatment with potassium/sodium citrate (metabolized to bicarbonate).

Both parents were healthy and originated from the Northeast region of Thailand. Consanguinity, growth retardation, and renal or bone disease were notably absent from the family history. Both parents and the unaffected child of normal stature had normal plasma bicarbonate values. The mother and the unaffected child each exhibited normal urinary acidification (to pH 5.2 and 5.3, respectively) after oral ammonium chloride loading. Thus, dRTA was clinically diagnosed as recessively transmitted in this family.

Hemoglobin electrophoresis revealed Hb E/E also in the unaffected sib (II:1) and mother (I:2) and Hb A/E in the father (I:1), but reticulocytosis and evidence of hemolysis were absent from unaffected family members. Red cells of the mother and affected son (II:2) showed similarly reduced osmotic fragility and microcytosis, in contrast to normal values in the father.

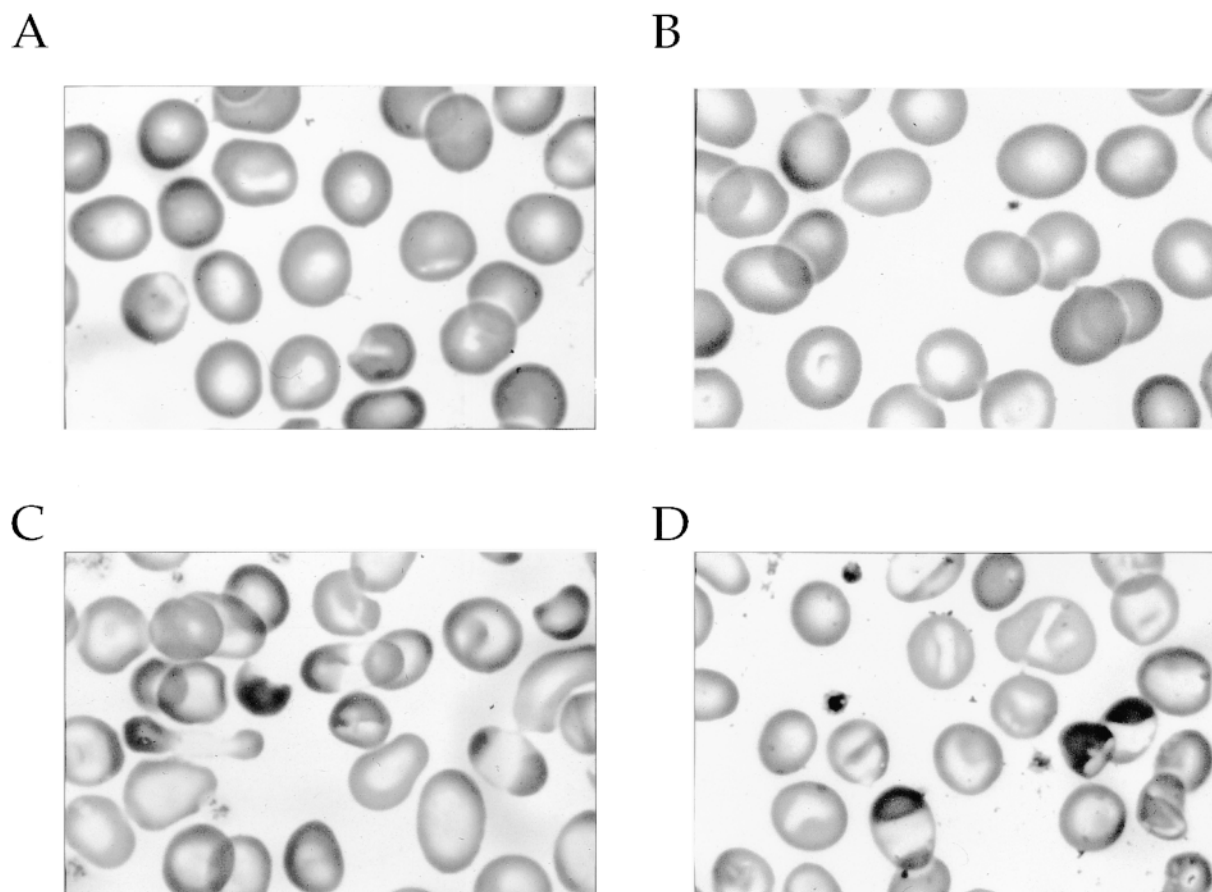


Figure 1. Peripheral blood smears (Wright's stain) from (A) mother I:2, (B) father I:1, (C) affected sib II:3, and (D) proband II:2 (Hb A/E). Target cells, spherocytes, stomatocytes, hypochromia, and anisopoikilocytosis in A, C, and D are due to Hb E/E status. Also present in C and D are xerocyte-like dumbbell forms, and cells with transverse ridges. These last features, along with more extreme microcytosis, basophilic stippling, and polychromasia, differ from typical Hb E/E blood smears.

Genetic analysis. Genomic DNA was extracted from peripheral blood leukocytes (SepaGene, Sanko Junyaku, Tokyo). *AE1* exons 2–20 (GenBank X77738 and L35930) were PCR-amplified using flanking intronic primers (primer sequences are available from authors upon request). PCR-amplified DNA fragments were subjected to single-strand conformational polymorphism (SSCP) analysis on mini-slab polyacrylamide gels at 18°C and visualized by silver stain (BioRad, Richmond, CA) (13). DNA fragments with abnormal mobilities were directly sequenced or were subcloned (pT7Blue; Novagen, Madison, WI) and ≥ 10 subclones of each were sequenced with an ABI 373A DNA sequencer (Prism Ready Reaction Dye Deoxy™ Terminator Cycle Sequencing kit; Applied Biosystems, Foster City, CA). Sequence variants that modified a restriction enzyme cleavage site were assayed in subsequent genomic DNA samples by restriction digestion of the appropriate exonic PCR product.

Analysis of erythrocyte membrane proteins. EDTA-anticoagulated blood from family members and normal controls was shipped on ice to Fukuoka, Japan or Boston, MA. 24–48 h after venipuncture, ghosts were prepared from washed erythrocytes by lysis in 50 vol ice-cold 5 mM Na-phosphate, pH 8.0, containing 0.2 or 1.0 mM phenylmethylsulfonyl-fluoride. Exofacial chymotryptic cleavage of AE1 for detection of the Memphis I polymorphism (14) was achieved by incubation of washed intact erythrocytes at 37°C for 30 min in 140 mM NaCl, 10 mM Na phosphate, pH 7.4, (PBS) containing 0.5 mg/ml chymotrypsin (Sigma, St. Louis, MO). *N*-deglycosylation of membranes with pepti-

dyl-*N*-glycosidase F (PNGase F; New England Biolabs, Beverly, MA) for 30 min at 37°C, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and densitometric scanning of Coomassie Blue R250–stained gels were as previously described (5).

Red cell anion transport studies. ³⁵S-sulfate influx studies in the presence and absence of the inhibitor, 4,4'-diisothiocyano-4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS; Sigma or Calbiochem, La Jolla, CA), were carried out within 48 h after venipuncture (5, 15).

Functional expression of recombinant AE1. Plasmids encoding human eAE1 in pBluscript KS, kAE1 in pXT7 containing the 5' and 3'-untranslated regions of *Xenopus* β -globin, protein 4.2 in pGEM-7Zf (gift from C. Korsgren and S.E. Lux, Harvard Medical School) and glyophorin A (GPA; M-allele) in pSG5 (gift from S.L. Spitalnik, University of Pennsylvania, Philadelphia, PA) were described previously (5, 16, 17). The G701D AE1 cDNA plasmid was constructed by four-primer PCR methods. The flanking primers were F1 (5'-GCC-ATGATGCTGCGCAAG-3', nt 1903–1920) and F2 (5'-ATAAGT-GCATGCGCCAGG-3', nt 2656–2639). The internal mutagenic primers were M1 (5'-GCTCCGACTTCCACCTGG-3', nt 2246–2263) and M2 (5'-CCAGGTGGAAGTCGGAGC-3', nt 2263–2246). The 754-bp PCR fragment was then cleaved by Fsp I and Sph I and subcloned into similarly cleaved human eAE1 and kAE1 recipient plasmids. The M31T/K56E eAE1 plasmid was constructed by reverse transcription (RT)-PCR from reticulocyte RNA of affected individual II:3. Primers used were 5'-GCCATGGAGGAGCTGC-3' (nt

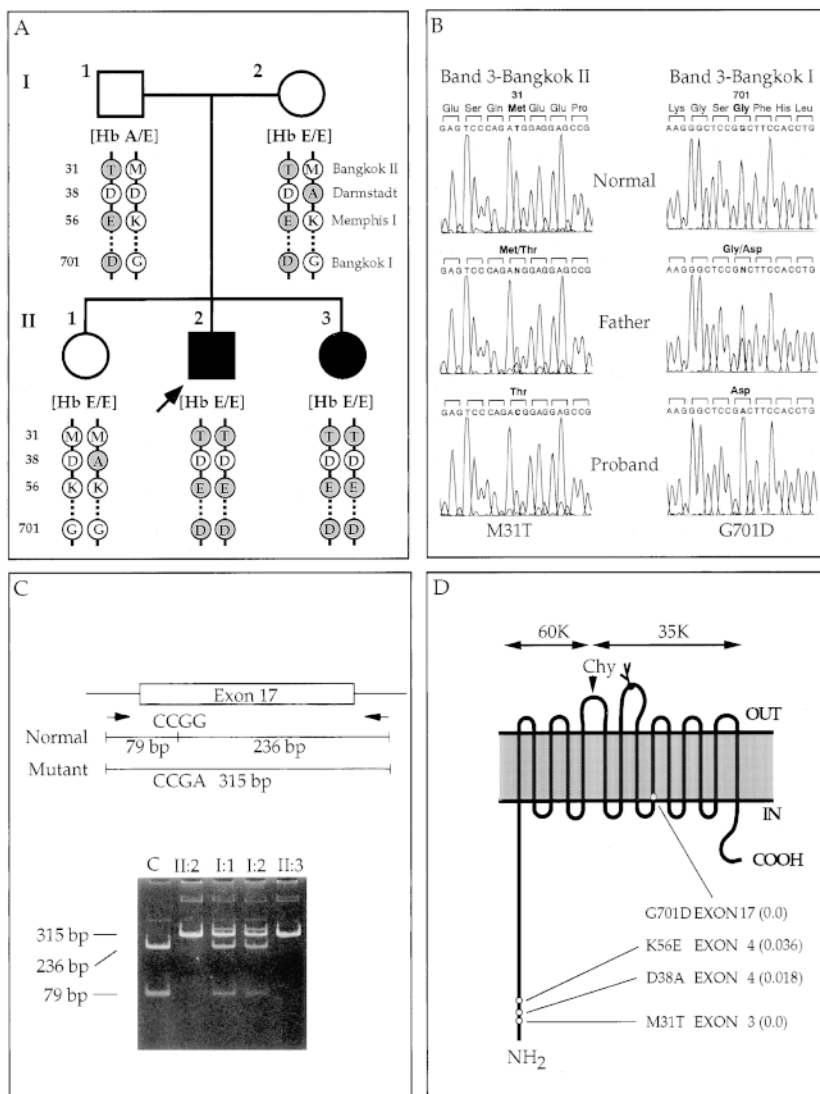


Figure 2. (A) Family pedigree. Proband is indicated by arrow, dRTA by filled symbols. Under each individual are the Hb genotype and the two AE1 alleles with predicted missense amino acid substitutions indicated by darkened symbols. Amino acid residue numbers are at left. City eponyms for the mutations are at upper right. (B) DNA sequence profiles of the regions adjacent to codons 31 and 701 for a normal control, the heterozygous individual I:1 and the homozygous proband II:2. (C) Restriction fragment length polymorphism analysis to screen for the G701D mutation used the abolition of an Msp I site by the Bangkok I mutation in the PCR-amplified fragment of exon 17. Control genomic DNA (C) is compared with that of indicated family members. (D) Codon substitutions encoded by the four mutations detected in the indicated exons of the AE1 gene of family members are mapped onto a topographic representation of human eAE1 polypeptide. Gene frequencies of the mutations among 55 normal, unrelated Thai individuals are shown within the adjacent parentheses. Human kidney AE1 (kAE1) initiates at Met 66 (9, 10) and so lacks codons 31, 38, and 56. The site of chymotryptic cleavage of eAE1 in intact red cells is indicated by the arrowhead (*Chy*), and generates the N-terminal fragments that can distinguish wild-type AE1 (60 kDa) from the K56E (Memphis I) variant (63 kDa).

148–163) and 5'-CTTTCCAGAATTCCAGATGG-3' (nt 807–787). The 660-bp PCR product was cleaved by Nco I and Eco RI, then subcloned into similarly cleaved eAE1 recipient plasmids of wild type and G701D type. Integrity of all constructs was confirmed by DNA sequence analysis. cRNAs were transcribed with the Megascript kit (Ambion, Austin, TX). cRNA was injected into defolliculated *Xenopus* oocytes of stages V–VI. Oocytes maintained in ND-96 buffer at 19°C for 2–3 d were subjected to ³⁶Cl⁻ influx studies as described previously (5).

Immunocytochemistry. Cryosections (6–8 μm) of *Xenopus* oocytes previously injected with AE1 cRNA plus or minus GPA cRNA were cut at -20°C and postfixed in acetone for 3 min. After 1 h preincubation with PBS/1% BSA, sections were incubated 1 h at 20°C with affinity-purified rabbit polyclonal anti-mouse AE2 antibody cross-reactive with the AE1 C-terminal dodecapeptide (18), washed thrice with PBS, and incubated 1 h with FITC-labeled goat anti-rabbit Ig (Jackson ImmunoResearch, West Grove, PA; 1:1,000). The sections were then washed, reblocked in PBS/1% BSA, incubated for 1 h at room temperature with mouse monoclonal anti-GPA antibody 10F/7 (16), washed, and stained with Cy3-labeled goat anti-mouse Ig (Jackson ImmunoResearch; 1:1,000). Specificity of the staining was tested in water-injected oocytes. Sections examined with an Olympus BH2 epifluorescence photomicroscope were photographed with Kodak Ektachrome 400 film.

Immunoprecipitation and cell surface biotinylation. Oocytes metabolically labeled for 2 d with ³⁵S-Translabel (ICN, Costa Mesa, CA) were extracted with 1% Triton X-100 and subjected to immunoprecipitation with mouse monoclonal anti-human AE1 antibody IVF12 (gift of V. Schuster, Albert Einstein College of Medicine, New York) as described previously, with slight modifications (19). To detect cell surface expression, ³⁵S-labeled oocytes were preincubated in 10 mM NaIO₄ at 4°C for 30 min, washed, and labeled at 4°C for 1 h with 2 mM biotin hydrazide (Sigma) in 100 mM NaOAc. Biotinylated oocytes were washed and quenched in a solution containing 5 mM glycine for 10 min, and whole cell extracts were subjected to AE1 immu-

noprecipitation. Immune complexes were released from Protein A Sepharose by incubation in acid glycine solution containing 1% Triton X-100 for 25 min at 20°C, and the neutralized supernatant was incubated 1 h at 4°C with avidin-agarose beads (Pierce, Rockford, IL). The beads were washed and resuspended in SDS sample buffer for 1 h at room temperature. Eluted immunoprecipitates were analyzed by SDS-PAGE and autoradiography.

Results and Discussion

Detection of AE1 mutations in genomic DNA. Though associations of familial dRTA with cosegregating AE1 mutations have been restricted thus far to cohorts displaying an autosomal dominant inheritance pattern, the combined erythroid and renal phenotype in this family recalled the spherocytosis and acidosis evident in a bovine cohort with a homozygous loss-of-function mutation in AE1 (20), and prompted evaluation of AE1 as the candidate disease gene in this family. SSCP analyses of exonic fragments of the AE1 gene identified anomalous conformers for exons 3, 4, and 17 among the five family members examined.

DNA sequencing revealed three cosegregating missense mutations. Band 3 Bangkok I changes codon 701 in exon 17 from GGC to GAC, ablating an Msp I site (Fig. 2 C), to encode the novel mutation G701D. Band 3 Bangkok II changes codon 31 in exon 3 from ATG to ACG to encode the novel mutation M31T. The third mutation is the known exon 4 polymorphism, band 3 Memphis I (K56E) (14, 21), also present in cis with Southeast Asian Ovalocytosis (22) and with numerous spherocytosis mutations (23, 24). Both affected individuals were homozygous for these three mutations, whereas both unaffected parents and the one unaffected sister were heterozy-

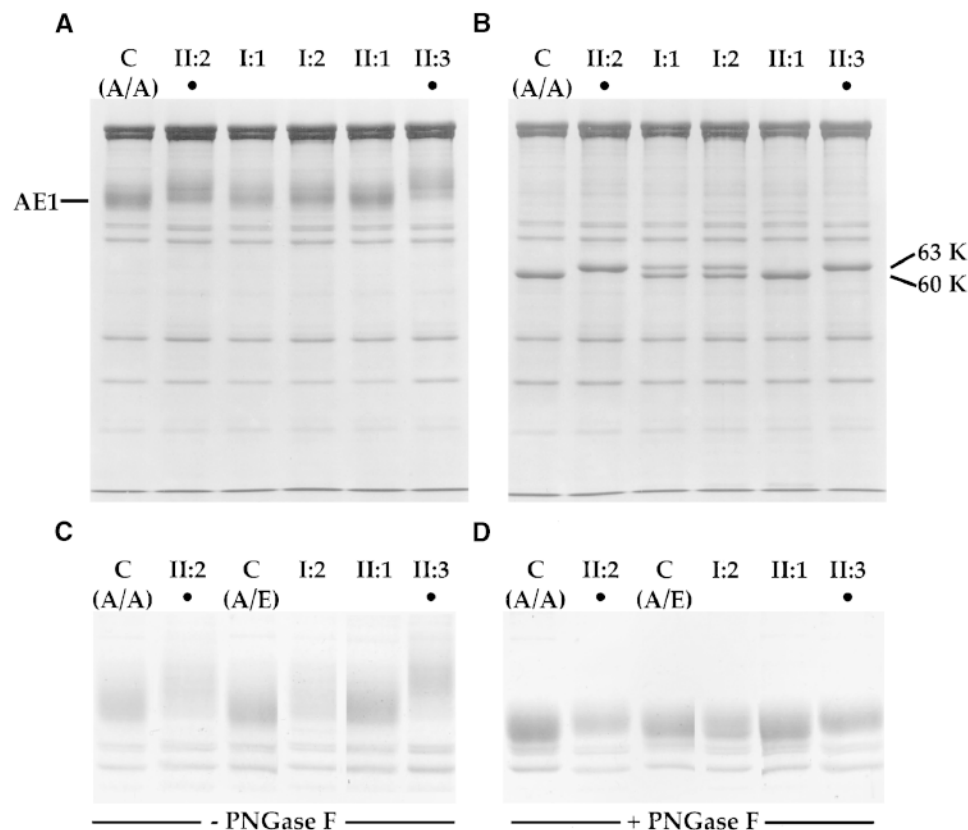


Figure 3. SDS-PAGE-separated, Coomassie Blue-stained red cell ghost polypeptides were prepared from intact red cells (A) or from cells previously treated with chymotrypsin (B). Ghosts from intact red cells were incubated in the absence (C) or presence of PNGase F (D). Filled circles above lanes indicate affected individuals.

gous (Fig. 2, A and B). The mother and unaffected child were also heterozygous in *trans* for the exon 4 polymorphism, band 3 Darmstadt (D38A) (25). Among these four mutations all expressed in eAE1, only G701D is expressed in the N-terminally truncated kAE1, and is the sole mutation located in the anion translocating transmembrane domain (Fig. 2 D). Neither mutation G701D nor M31T was detected in the *AE1* genes of clinically normal, unrelated individuals of Thai ($n = 55$) or Japanese origin ($n = 48$). Thai gene frequencies ($n = 55$) were 0.036 for Memphis I variant K56E and 0.118 for Darmstadt variant D38A. Japanese gene frequencies were 0.110 for K56E ($n = 123$) and 0.125 ($n = 36$) for D38A (26).

Effects of *AE1* mutations on red cells. Fig. 3 shows that unaffected and affected family members had comparable contents of AE1 polypeptide (A), with M_r that varied slightly according to Memphis I (K56E) status (14, 21), as further revealed by chymotryptic digestion of intact red cells (B). *N*-deglycosylation of AE1 in intact red cells (C) did not reveal evident differences in *N*-glycan content (D). In addition, DIDS-sensitive sulfate influx in red cells of affected individuals II:2 and II:3 (33.4 and 27.4 mmol/10¹³ cell × h) did not differ from that in Hb E/E cells from the unaffected mother I:2, unaffected child II:1, or from control (Hb A/E) cells (33.0, 32.2, and 35.5 mmol/10¹³ cell × h, respectively). The findings suggest little or no effect of these AE1 mutations on red cell eAE1 anion transport phenotype under the conditions tested.

Functional expression of mutant AE1 polypeptides in *Xenopus* oocytes. The lack of impaired anion transport in red cells of affected family members differed from the anion transport phenotype of mutant AE1 polypeptides in cRNA-injected *Xenopus* oocytes (Fig. 4). Consistent with the clinical renal phenotype, recombinant kAE1 G701D function was reduced to 1.7% and 2.4% of wild-type values assayed at 20 and 37°C, respectively (Fig. 4 A). The relative transport activities of wild-type and mutant kAE1 were unchanged in acidic (pH 5.5) or hypertonic medium (not shown). However, recombinant eAE1 G701D function in *Xenopus* oocytes was similarly reduced to 11.7% (20°C) and 7.2% (37°C) of wild-type values, in contrast to the unimpaired transport function in red cells.

To reconcile the mild red cell phenotype with the severe oocyte phenotype of eAE1 G701D, the two additional eAE1 mutations were examined. As shown in Fig. 4 B, the eAE1 double mutant M31T/K56E did not differ in function from wild-type eAE1, nor did the triple mutant M31T/K56E/G701D exhibit any greater transport activity than did the single mutant G701D. Consistent with the recessive clinical phenotype, none of the AE1 mutants exhibited dominant negative properties in oocytes when coexpressed with wild-type AE1 (Fig. 4 C).

Rescue of the loss-of-function phenotype of the *dRTA* mutation. The loss-of-function phenotype of AE1 G701D in *Xenopus* oocytes can account for the patients' urinary acidification defects but contrasts with their apparently normal red cell sul-

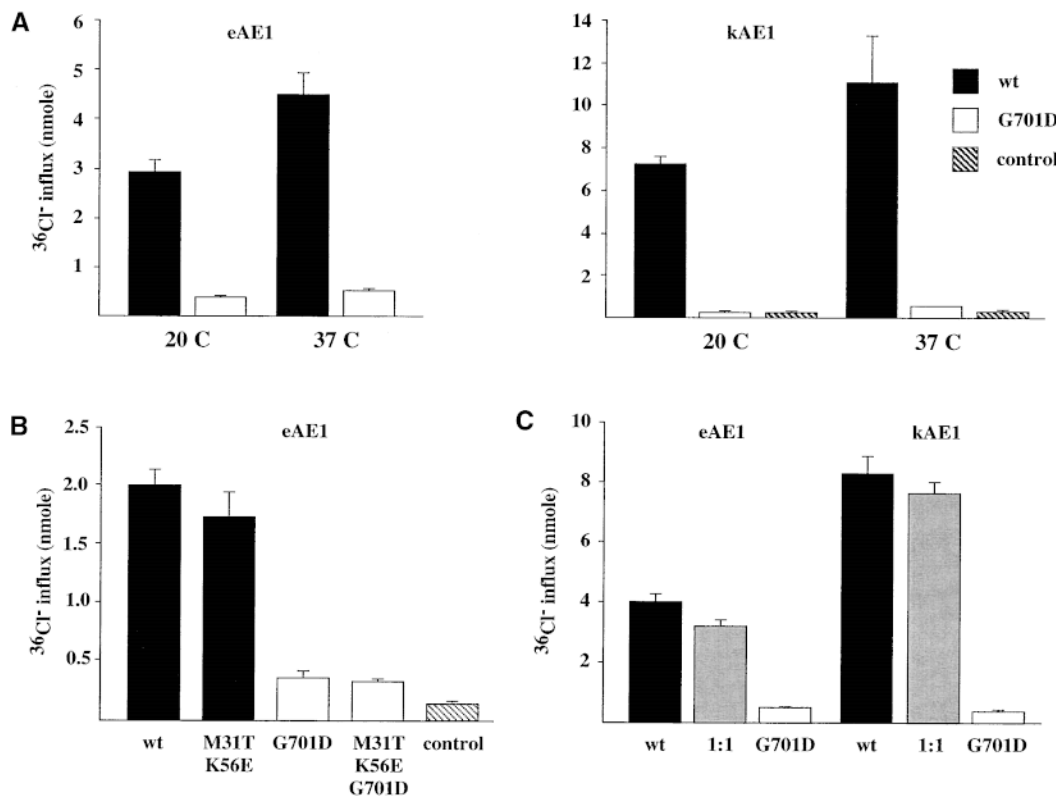


Figure 4. Functional effect of the G701D AE1 mutation. *Xenopus* oocytes previously injected with water or with cRNA encoding the indicated AE1 polypeptides were subjected to 15 min $^{36}\text{Cl}^-$ influx assays. (A) Comparison of $^{36}\text{Cl}^-$ uptake mediated by wild-type and G701D forms of eAE1 and kAE1 in isotonic medium, pH 7.4, at 20 or 37°C, as indicated. (B) Comparison of eAE1-mediated $^{36}\text{Cl}^-$ uptake at 20°C by wild-type, M31T/K56E, G701D, and triple mutant M31T/K56E/G701D polypeptides. (C) Comparison of $^{36}\text{Cl}^-$ influx into oocytes expressing wild-type AE1, AE1 G701D, and 1:1 mixtures of the two indicated no dominant negative effects of G701D AE1. Values are means \pm SEM of uptake pooled from five experiments with six to eight oocytes per group in (A) and two to three experiments in (B) and (C), respectively. $P < 0.0001$ comparing wild-type with G701D AE1.

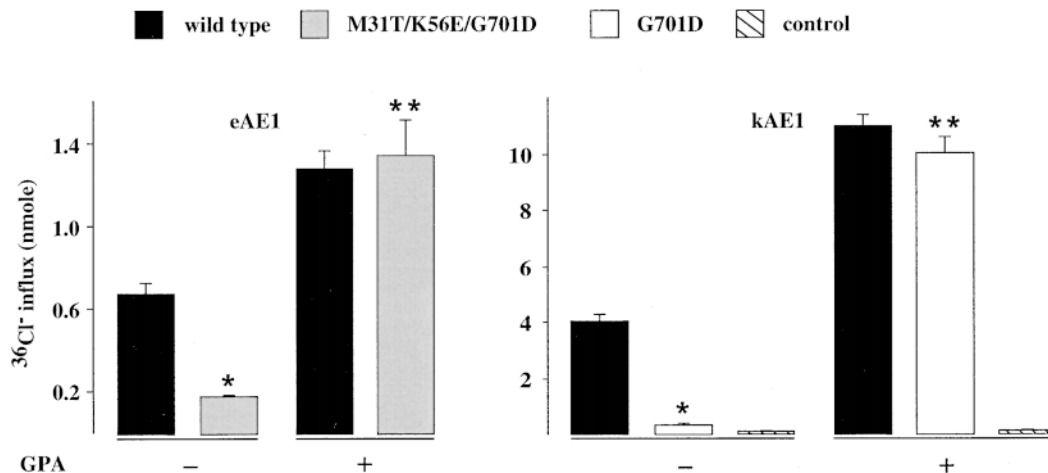


Figure 5. Functional rescue of AE1G701D loss-of-function mutation by coexpression of GPA. Oocytes were injected with either AE1 alone (1.5 ng cRNA) or AE1 plus GPA (1.5 ng, both cRNAs), and $^{36}\text{Cl}^-$ uptake was measured 3–4 d later. The data are represented as means \pm SEM from two or three separate experiments with eAE1 and kAE1, respectively (six to eight oocytes per experiment). * $P < 0.0001$ compared with wild-type AE1; ** $P > 0.05$ compared with wild-type AE1 plus GPA.

fate transport. eAE1 is known to bind to several red cell proteins, including hemoglobin, glycolytic enzymes, ankyrin1, protein 4.2, and GPA (7). Complete deficiencies of protein 4.2 in mouse (27) and human erythrocytes (H. Ideguchi, C. Shayakul, S.L. Alper, and C. Brugnara, unpublished data) and of GPA in human erythrocytes (28) have been associated with decreased AE1 protein and AE1-mediated sulfate transport.

Therefore, the hypothesis was tested that the difference between AE1 G701D function in oocytes and in erythrocytes reflects the different complements of AE1-binding or chaperonin proteins in these cell types. Coexpression of erythroid protein 4.2 did not alter loss-of-function phenotype of eAE1 G701D (not shown). In contrast, the transport activity of AE1 G701D was completely rescued by GPA. Cl^- influx mediated by either the eAE1 triple mutant M31T/K56E/G701D or by the kAE1 single mutant G701D was indistinguishable from that of wild-type eAE1 or of kAE1 in the presence of coexpressed GPA (Fig. 5).

Previous studies have shown that coexpression of GPA with wild-type AE1 accelerates, but is not required for, accumulation of AE1 polypeptide at the oocyte surface (29). Thus, it is plausible that a similar mechanism might explain the phenotypic rescue by GPA of AE1 G701D. Immunofluorescence (Fig. 6, A–D) and cell surface biotinylation studies (Fig. 6 E) revealed much reduced plasma membrane accumulation of AE1 G701D polypeptide compared with that of wild-type AE1. Total accumulation of G701D AE1 was also lower than that of wild-type AE1 after injection of limiting amounts of cRNA (Fig. 6 E), but this difference was less marked at high AE1 cRNA concentrations (not shown). GPA coexpression increased both total and surface-associated AE1 G701D polypeptide to wild-type levels (Fig. 6, D–E), consistent with the red cell phenotype. GPA levels in red cells of both affected individuals were normal as determined by immunoblot (not shown).

These data indicate that the GPA–AE1 interaction, seemingly optional for wild-type AE1, is rendered essential by the AE1 G701D mutation. Though GPA is expressed in the red cell and GPA mRNA can be detected by RT-PCR in reticulocyte RNA, RT-PCR fails to detect GPA mRNA in human kidney (data not shown). Thus, we postulate that in type A inter-

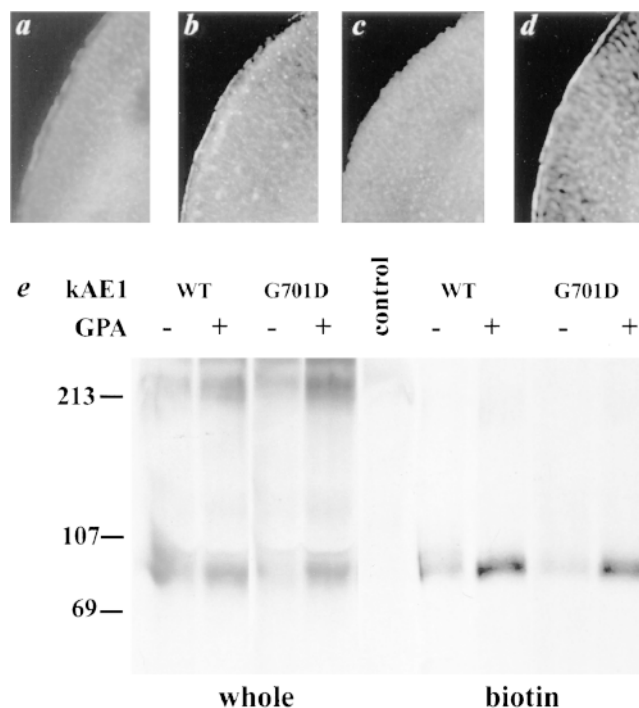


Figure 6. AE1 polypeptide expression in *Xenopus* oocytes and influence of coexpressed GPA. Immunofluorescence localization of AE1 in the plasma membrane of oocytes expressing wild-type kAE1 (a, minus GPA; b, plus GPA) and kAE1 G701D (c, minus GPA; d, plus GPA). Sectioning, fixation, incubations, and photomicroscopy were performed under identical conditions. GPA staining intensity at the oocyte surface was equivalent in the presence of wild-type and mutant kAE1 (not shown). Representative of three similar experiments. (e) Expression level of wild-type kAE1 and kAE1 G701D polypeptide in whole oocytes and at the cell surface in the absence and presence of coexpressed GPA. First four lanes show autoradiograph of whole cell lysates from metabolic labeled oocytes after AE1 immunoprecipitation (~ 5 oocyte equivalents/lane). Last four lanes show products from metabolic labeled oocytes subjected to cell surface biotinylation, AE1 immunoprecipitation and sequential avidin-agarose precipitation (30 oocyte equivalents per lane). Oocytes were injected with water (control) or 1.5 ng AE1 cRNA \pm 1.5 ng GPA cRNA. One of two similar experiments.

calated cells lacking GPA, the AE1 G701D mutation produces a conditional phenotype of impaired biosynthetic trafficking and/or protein stability that can be rescued by GPA coexpression.

Elucidation of the mechanism by which the triply mutant AE1 contributes to hemolytic anemia in this family with homozygous HbE will require further investigation.

In summary, autosomal recessive dRTA in this single, small kindred, in contrast to 17 previously reported recessive or possibly recessive kindreds (6), cosegregates with and is very likely caused by homozygosity for the AE1 mutation G701D. The functional data in oocytes suggest that the G701D mutation leads to decreased or absent kAE1 accumulation at the basolateral plasma membrane of type A IC in the renal collecting duct. The minimal effect of this pathological mutation on red cell eAE1 abundance and sulfate transport likely reflects the presence in red cells of the eAE1 chaperonin, GPA. This is the first reported case of recessive dRTA cosegregating with mutant *AE1* and the first in which the functional consequences of the mutation can explain the urinary acidification phenotype. Functional rescue of this conditional *AE1* mutation also suggests new therapeutic routes to pharmaceutical and gene therapy of dRTA.

Acknowledgments

We thank Michelle Rotter, Guido Buchbinder, and Alan Stuart-Tilley for technical assistance, and Leah Staffier for administrative assistance.

This work was supported by National Institutes of Health Grants DK43495 (S.L.A.), HL15157 (C.B. and S.L.A.), and DK34854 (Harvard Digestive Diseases Center to S.L.A.). S.L.A. is an Established Investigator of the American Heart Association.

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