REVIEW

Molecular mechanisms of autosomal dominant and recessive distal renal tubular acidosis caused by *SLC4A1 (AE1)* mutations

Pa-thai Yenchitsomanus[1,2,3,*], Saranya Kittanakom[4], Nanyawan Rungroj[3], Emmanuelle Cordat[4], Reinhart A F Reithmeier[4]

[1]Division of Medical Molecular Biology and [2]BIOTEC-Medical Biotechnology Unit, [3]Division of Molecular Genetics, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. [4]Departments of Biochemistry and Medicine, University of Toronto, Toronto, Ontario, M5S 1A8, Canada

[*]*Correspondence to*: Pa-thai Yenchitsomanus, *Email*: grpye@mahidol.ac.th, *Tel*: +662 4197000 ext 6666-70, *Fax*: +662 4184793

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ABSTRACT

Mutations of *SLC4A1 (AE1)* encoding the kidney anion (Cl^-/HCO_3^-) exchanger 1 (kAE1 or band 3) can result in either autosomal dominant (AD) or autosomal recessive (AR) distal renal tubular acidosis (dRTA). The molecular mechanisms associated with *SLC4A1* mutations resulting in these different modes of inheritance are now being unveiled using transfected cell systems. The dominant mutants kAE1 R589H, R901X and S613F, which have normal or insignificant changes in anion transport function, exhibit intracellular retention with endoplasmic reticulum (ER) localization in cultured non-polarized and polarized cells, while the dominant mutants kAE1 R901X and G609R are mis-targeted to apical membrane in addition to the basolateral membrane in cultured polarized cells. A dominant-negative effect is likely responsible for the dominant disease because heterodimers of kAE1 mutants and the wild-type protein are intracellularly retained. The recessive mutants kAE1 G701D and S773P however exhibit distinct trafficking defects. The kAE1 G701D mutant is retained in the Golgi apparatus, while the mis-folded kAE1 S773P, which is impaired in ER exit and is degraded by proteosome, can only partially be delivered to the basolateral membrane of the polarized cells. In contrast to the dominant mutant kAE1, heterodimers of the recessive mutant kAE1 and wild-type kAE1 are able to traffic to the plasma membrane. The wild-type kAE1 thus exhibits a 'dominant-positive effect' relative to the recessive mutant kAE1 because it can rescue the mutant proteins from intracellular retention to be expressed at the cell surface. Consequently, homozygous or compound heterozygous recessive mutations are required for presentation of the disease phenotype. Future work using animal models of dRTA will provide additional insight into the pathophysiology of this disease.

KEYWORDS: *Distal renal tubular acidosis, kidney anion exchanger, protein trafficking, dominant negative effect, dominant positive effect*

INTRODUCTION

Primary distal renal tubular acidosis (dRTA) is a clinical syndrome characterized by impairment of acid secretion by α-intercalated cells in the distal nephron and collecting tubule, resulting in decreased net acid excretion and inability of kidney to lower urinary pH <5.5 in spite of spontaneous academia or after acid loading (Batlle et al 2001; Alper , 2002; Herrin, 2003). The decreased net acid excretion attributable to decreased ammonium and titratable acid excretion leads to a positive acid balance and hyperchloremic metabolic acidosis. The presence of chronic

metabolic acidosis consequently decreases renal excretion of citrate (hypocitraturia) while increases calcium excretion (hypercalciuria), creating a favorable environment for urinary stone formation and nephrocalcinosis. The other associated abnormalities include hypokalemia leading to muscle weakness, and metabolic bone disease (rickets or osteomalacia). The disease can be caused either by a defect in the H^+-ATPase, which is responsible for acid (H^+) secretion at the apical membrane of the epithelial α-intercalated cells or by an abnormality of the kidney anion exchanger 1 (kAE1) that functions in chloride (Cl^-) and bicarbonate (HCO_3^-) anion exchange at the basolateral membrane (Figure 1). The defects of these two proteins may occur from mutations of the responsible genes causing hereditary dRTA. Mutations of at least two genes encoding two subunits (B1 and a4) of H^+-ATPase have been reported to cause autosomal recessive dRTA with sensorineural deafness or normal hearing (Karet et al, 1999; Smith et al, 2000; Stover et al, 2002; Ruf et al, 2003). The genes that encode B1 subunit in V_1 (catalytic) domain and a4 subunit in Vo (transmembrane) domain of H^+-ATPase are *ATP6V1B1* (MIM 192132) located on chromosome 2p13.1 and *ATP6V0A4* (MIM 605239) situated on chromosome 7q33-q34, respectively. Also, mutations in human *solute carrier family 4, member 1 (SLC4A1)* or *anion exchanger 1 (AE1)* gene (MIM 109270) may cause dRTA. *SLC4A1* located on chromosome 17q21-q22 encodes both erythroid (eAE1, band 3) and kidney (kAE1) isoforms of AE1 protein. Thus, *SLC4A1* mutations shows pleiotrophic effects resulting in two distinct and seemingly unrelated disorders, red cell abnormalities (e.g., hereditary spherocytosis and ovalocytosis) and dRTA.

An abnormality of kAE1 that mediates chloride/bicarbonate (Cl^-/HCO_3^-) exchange at the basolateral membrane of the α-intercalated cells can lead to a defect in bicarbonate (HCO_3^-) extrusion across this membrane and to its intracellular accumulation. To maintain intracellular acid-base and electroneutal balances, the cells would reserve acid or hydrogen ion (H^+) instead of secreting through the apical membrane. Consequently, the intracellular accumulation of both bicarbonate and hydrogen ions would inhibit the dissociation of carbonic acid (H_2CO_3) (Figure 1). The failure of hydrogen ion (H^+) secretion through the apical membrane into the tubular lumen due to a kAE1 defect will eventually result in dRTA.

It was previously unknown why mutations of *SLC4A1* cause both autosomal dominant (AD) (Bruce et al, 1997; Jarolim et al, 1998; Karet et al, 1998; Weber et al, 2000; Sritippayawan et al, 2003; Cheidde et al, 2003) and autosomal recessive (AR) dRTA (Tanphaichitr et al, 1998; Vasuvattakul et al, 1999; Bruce et al, 2000; Ribeiro et al, 2000; Yenchitsomanus et al, 2002; Sritippayawan et al, 2004). During the past several years, several groups have begun to unravel the molecular mechanisms of dRTA caused by *SLC4A1* mutations presenting in these two different manners of inheritance. We have recently demonstrated that dominant and recessive dRTA phenotypes associated with *SLC4A1* mutations are determined by different behaviors in the intracellular trafficking of heterodimers between mutant and wild-type kAE1. The dominant kAE1 mutants result in intracellular retention of the wild-type kAE1, the so called 'dominant-negative effect', whereas the wild-type kAE1 exhibits 'dominant-positive effect' relative to the recessive mutant kAE1 because it can rescue the recessive mutant kAE1 to express on the cell surface. We have therefore distinguished the molecular mechanisms of AD and AR dRTA caused by *SLC4A1* mutations.

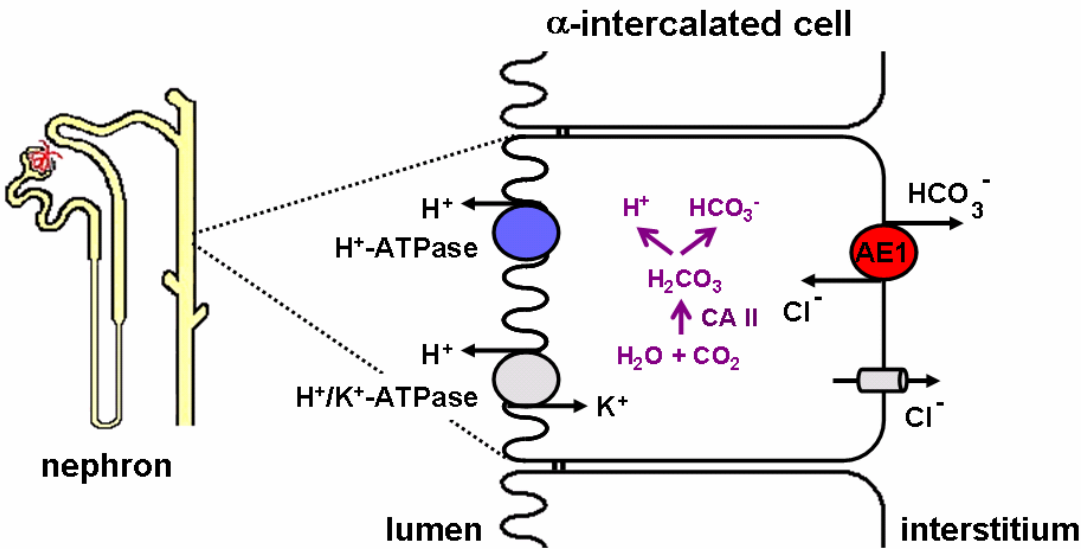

Figure 1. Schematic diagram of the α-intercalated cell in the distal nephron. H^+-ATPase and H^+/K^+-ATPase, involving in acid (H^+) secretion (in exchange with K^+ in the latter), are present at the apical membrane, while kAE1, functioning in chloride/bicarbonate (Cl^-/HCO_3^-) exchange, is located at the basolateral membrane. H^+ and HCO_3^- for apical secretion and basolateral reabsorption are dissociated from H_2CO_3, generated from hydration of carbon dioxide (CO_2), which is catalyzed by carbonic anhydrase (CA) II.

SLC4A1 AND ENCODED PROTEINS

SLC4A1

The human *SLC4A1* or *AE1* gene encoding the anion exchanger 1 (AE1 or band 3) is located on chromosome 17q21-q22 encompassing approximately 20 kb and consisting of 20 exons separated by 19 introns. The gene contains no TATA or CCAAT boxes in its upstream region (Tanner et al, 1988; Lux et al, 1989; Sahr et al, 1994) but consists of transcription factor consensus binding sites, activator protein 1 (AP1), activator protein 2 (AP2), CACCC boxes, GATA (erythroid factor 1), and E-boxes in this region. Instead, the TATA and CCAAT boxes are found in intron 3. The structure of the gene showed extensive similarity to that of mouse *AE1*, with a high degree of conservation of exon/intron junctions (Sahr et al, 1994; Schofield et al, 1994). The human *SLC4A1* encodes both eAE1 and kAE1 isoforms by using different promoters and alternative splicing. While eAE1 mRNA is transcribed from all exons by using its upstream promoter, kAE1 mRNA is transcribed by using the promoter in intron 3. The eAE1 cDNA sequence comprises 4,906 nucleotides (nt), excluding its poly (A) tail. The kAE1 mRNA lacks the sequences of exons 1-3 of the eAE1 transcript but contains a part of intron 3 (designated as exon K1) in its 5' untranslated region (5' UTR) and the complete exon 4-20 sequences with the translation initiation (ATG) site for methionine (Met) at codon 66 (Kollert-Jöns et al, 1993). The downstream end of exon 20 is defined as the RNA cleavage site or poly A addition site. A consensus poly A signal (AATAAA) is located 20 nucleotides upstream of the RNA cleavage site.

Expression and function of eAE1 and kAE1

The human eAE1 or band 3 is a multifunctional polytopic membrane glycoprotein containing 911 amino acids (Tanner et al, 1988; Lux et al, 1989) with molecular weight (MW) of approximately 95 kDa carrying a single glycosylation site at Asn642. It is expressed on erythrocytes at a high copy number of approximately $1x10^6$ copies per cell and exists in mature red cells as a mixture of dimers and tetramers. The eAE1 tetramers bind to ankyrin and cytoskeleton proteins, a binding which is critical for maintenance of the biconcave disc shape of red blood cells. In ankyrin-deficient cells, AE1 is present mainly as dimers (Jennings, 1984). In peripheral tissues, eAE1 functions in electroneutral anion (Cl^-/HCO_3^-) exchange by transporting bicarbonate (HCO_3^-) out of erythrocytes in exchange for chloride (Cl^-) uptake into the cell, but it operates in the reverse mode in the lung. kAE1 which lacks 65 N-terminal amino-acids of eAE1, is expressed at the basolateral surface of the α-intercalated cells in the distal nephron (Alper et al, 1989; Kollert-Jöns et al, 1993). It provides a major exit route for HCO_3^- in exchange for Cl^- across the basolateral membrane of the α-intercalated cells, while hydrogen ion (H^+) is secreted through the apical membrane of these cells into the tubular lumen.

Structure of eAE1 and kAE1

eAE1 comprises two main domains that are structurally and functionally distinct. The 40-kDa N-terminal cytoplasmic domain, residues 1-360, involved in functions unrelated to anion transport, but acts as a membrane anchorage site for the red cell skeleton through the interactions with ankyrin, band 4.1, and band 4.2. This domain of eAE1 also binds to the glycolytic enzyme complex (Campanella et al, 2005). The N-terminal cytoplasmic domain of kAE1, 65 amino acids shorter than that of eAE1, does not bind to ankyrin or glycolytic enzymes (Wang et al, 1995; Zhang et al, 2000) and its interacting proteins in the α-intercalated cells remain to be identified. The 50-kDa C-terminal integral membrane domain with 12-13 transmembrane (TM) spans, from residues 361 to 882, mediates in Cl^-/HCO_3^- exchange (Groves and Tanner, 1999). The anion transport is inhibited by 4, 4'-diisothiocyanostilbene-2-2', disulfonic acid (DIDS), which covalently binds to extracellular Lys539 in TM5 and can crosslink to Lys851 in TM12 (Kawano et al, 1988; Okubo et al, 1994). Both eAE1 and kAE1 has a short acidic cytoplasmic C-terminal tail (residues 883-911), containing binding sites for carbonic anhydrase (CA) II (Vince and Reithmeier, 1998; Reithmeier, 2001) and for protein complexes involving in the targeting of kAE1 to the basolateral membrane of kidney α-intercalated cells (Devonald et al, 2003a; Toye et al, 2004).

AE1 – a transport metabolon

The short acidic cytoplasmic C-terminal tail (residues 883-911) of eAE1 and kAE1 binds to CA II. This binding facilitates and maximizes bicarbonate transport by channeling substrate from CAII directly to AE1 (Reithmeier RAF, 2001; Sterling et al, 2001). Bicarbonate (HCO_3^-) from the breakdown of carbonic acid (H_2CO_3) generated from hydration of carbon dioxide (CO_2) by CAII is transported across the cell membrane in exchange for chloride (Cl^-) by AE1. It was demonstrated in transfected human embryonic kidney (HEK) 293 cells that the binding between the cytoplasmic C-terminal region of AE1 and CAII provides maximal anion transport activity of AE1 (Sterling et al, 2001; Sterling and Casey, 2002). The binding motif of AE1 is acidic $L_{886}DADD_{890}$, interacting with a basic histidine cluster at the N-terminus of CAII (Vince and Reithmeier, 1998; Vince et al, 2000). Leu886 on AE1 is highly conserved among anion exchangers (Vince and Reithmeier, 1998) and important for the binding, suggesting the involvement of a hydrophobic amino acid residue in the interaction. The binding motif close to the membrane would ideally position CAII for coordinating its function with AE1. The association of CAII – a soluble enzyme and AE1 – a membrane transporter is regarded as an example of a functional transport 'metabolon' (Reithmeier, 2001), a weakly associated protein complex involved in a metabolic and transporting pathway to facilitate movement of metabolite directly from one protein to the next. Treatment of AE1-transfected HEK 293 cells with the CA inhibitor, azetazolamide, gave rise to almost complete inhibition of anion transport activity, and blocking of CAII binding to AE1 resulted in a decrease in Cl^-/HCO_3^- transport (Sterling et al, 2001).

Targeting of eAE1 and kAE1

Trafficking of eAE1 from its biosynthesis site and intracellular compartments to the red cell membrane is facilitated by glycophorin A (GPA), a 36 kDa glycoprotein of the red cell membrane (Bruce et al, 1994). The importance of the N-terminus of kAE1 in protein targeting has recently been described (Toye et al, 2004). In

stably transfected Madin-Darby canine kidney (MDCK) cells expressing the AE1 membrane domain (residues 361-911), lacking the N-terminal domain, the protein was localized to the apical membrane, suggesting that elements within the N-terminal sequence are required for the basolateral targeting of kAE1. $Y_{359}KGL_{362}$ is a potential tyrosine-targeting motif in the N-terminus of kAE1.

A motif ($Y_{904}DEV_{907}$) is present on the cytoplasmic C-terminal tail of human AE1. This motif conforms to a subset of tyrosine-based signals, YXXØ motif (where Y is tyrosine, X is any amino acid, and Ø is hydrophobic amino acid) (Canfield et al, 1991), which is known to be involved in localization to coated pits and clathrin-mediated endocytosis, and also in basolateral sorting (Matter et al, 1994; Lin et al, 1997; Distel et al, 1998). Many membrane proteins are usually sorted to the basolateral surface through interactions of tyrosine-based signal motifs in their cytoplasmic domains with adaptor-protein complexes (Bonifacino and Dell'Angelica, 1999; Folsch et al, 1999). This motif plays an important role in kAE1 distribution in polarized cells. Tyr904 in the YXXØ motif of kAE1 has been examined for the basolateral signal in MDCK cells and rat inner medullary collecting duct (IMCD) cells (Devonald et al, 2003a; Toye et al, 2004). kAE1 with substitution of Tyr904 by alanine (Y904A) or with an 11 amino acid deletion (R901X) is mistargeted to the apical membrane (Devonald et al, 2003a; Toye et al, 2004). The YXXØ motif is recognized by the μ subunit of the adaptor protein (AP) complexes of AP-1, AP-2, AP-3, and AP-4, (Ohno et al, 1995; Bonifacino and Dell'Angelica, 1999; Bonifacino and Traub, 2003), especially AP-1B which is specific to polarized epithelial cells (Ohno et al, 1999; Folsch et al, 1999). Nevertheless, AP-1B may not be involved in kAE1 targeting as shown in a study using renal epithelial cell line from porcine kidneys (LLC-PK1) lacking the μ1B subunit (Devonald et al, 2003a; Toye et al, 2004).

The acidic patch (DE) at positions 905-906 following Tyr904 and the last 4 amino acids ($A_{908}MPV_{911}$) at the C-terminus may also be required for trafficking and localization of kAE1. The $A_{908}MPV_{911}$ motif has been proposed as a potential PDZ protein binding domain (XØXØ; where Ø represents a hydrophobic amino acid). Replacement of the acidic patch (DE) with two alanines (AA), or deletion of the last 4 amino acids at the C-terminus (AE1Δ908-911) showed non-polarized distribution to apical membrane and some intracellular localization in transfected polarized MDCK and IMCD cells (Devonald et al, 2003b). A similar result was obtained from the trafficking study of the deletion of 5 amino acids at the C-terminus of AE1 (AE1Δ907-911) in transiently transfected HEK 293 cells and non-polarized LLC-PK1 cells (Cordat et al, 2003). The motifs present in the C-terminal tail of kAE1 may also be involved in endocytosis and retention in the basolateral membrane via interaction with the actin cytoskeleton. However, the proteins that interact with these motifs at the C-terminus of kAE1 in the α-intercalated cells have not yet been identified.

HUMAN DISEASES CAUSED BY *SLC4A1* MUTATIONS

Hereditary spherocytosis and dRTA

Mutations in eAE1 can result in hereditary spherocytosis (HS), a common inherited hemolytic anemia with the presence of osmotically fragile spheroidal-shape erythrocytes and splenomegaly (Bruce and Tanner, 1999). Approximately 20% of HS cases are caused by dominantly inherited heterozygous *SLC4A1* mutations (Jarolim et al, 1996; Bruce and Tanner, 1996; Tanner MJ, 1997; Tse and Lux, 1999). The link between AE1-deficient HS and dRTA was investigated (Rysava et al, 1997) and it was found that the patients with AE1-deficient HS had no dRTA although two out of eight patients studied had an incomplete form of dRTA, defined by acid secretion defect after an acid load (urine pH >5.5) but absence of clinical manifestation. These two patients, mother and daughter, carried a previously identified *SLC4A1* mutation causing band 3 PRIBRAM, a substitution of G by T at the position +1 of intron 12 (IVS12+1G>T), leading to the retention of intron 12 sequence following the normal codon 477 and encoding 7 novel amino acids and a termination condon (TGA) at the position 8th triplet of intron 12 (Jarolim et al, 1996). This has led to the conclusion that *SLC4A1* HS mutations are not normally sufficient to cause full expression of dRTA (Rysava et al, 1997). However, severe HS and dRTA with complete absence of eAE1 was found in the patient with homozygous *SLC4A1* V488M mutation (band 3 Coimbra) (Ribeiro et al, 2000).

The spectrum of AE1 deficiency in HS ranges from 20–40% decrease to complete absence of red cell AE1 content. These usually occur from nonsense, frameshift, and missense mutations of *SLC4A1* (Iolascon, et al 1998). The missense mutations may cause a greater decrease in AE1 expression in red cells, probably due to a dominant-negative effect (Dhermy et al, 1999). Several *SLC4A1* mutations that alter amino acids in the cytoplamic domain of AE1 may change binding sites for ankyrin, band 4.2, α- and β-spectrins (Eber et al, 1996; Alloisio et al, 1997). Additionally, the *SLC4A1* E40K and P327R mutations affecting the cytosolic domain of AE1 are associated with a decreased band 4.2 without loss of AE1 content (Jarolim et al, 1992; Rybicki et al, 1993). From studies in AE1 knock-out mice, it has been hypothesized that AE1 deficiency causes reduction of protein–lipid and protein–protein interactions in the red cell membrane, weakening the connections between the membrane and cytoskeleton and thereby causing blebbing of AE1-free portions of membrane (Peters et al, 1996). This would cause the reduction of surface area to volume ratio and the spheroidal shape of red cells.

The effect of seven *SLC4A1* HS-missense mutations (L707P, R760Q, R760W, R808C, H834P, T837M, and R870W), located within the transmembrane domains of AE1, on the biosynthesis and functional expression of AE1 in transfected HEK 293 cells, has been examined (Quilty and Reithmeier, 2000). All seven HS mutations caused the AE1 protein to mis-fold and be retained intracellularly. However, there was no change in the oligomeric state or the half-life of the mutant AE1. Intracellular retention of HS mutant AE1 would lead to destruc-

tion of the protein during erythroid development and would account for the lack of HS mutant AE1 in the plasma membrane of the mature red cell. The possible explanation for lack of the dRTA phenotype of *SLC4A1* HS mutations is that while these mutations affect eAE1 in the erythroid cells, they may have little or no effect on kAE1 in the kidney α-intercalated cells. However, it is also likely that some mutations may result in both HS and AR dRTA, which in the heterozygous condition would result in HS phenotype without dRTA symptoms. The combined HS and dRTA symptoms may only appear in patients carrying two mutant alleles. Homozygous *SLC4A1* V488M mutation causing severe HS and dRTA (Ribeiro et al, 2000) is a good example that supports this explanation.

Southeast Asian ovalocytosis and dRTA

Southeast Asian ovalocytosis (SAO) is a morphological red cell abnormality caused by a mutational deletion of 27 base-pair (bp) in exon 11 of *SLC4A1* leading to an in-frame 9 amino acid deletion involving Ala400-Ala408 of eAE1, at the junction between the N-terminal domain and the first transmembrane span (Jarolim et al, 1991). The mutant protein is inactive for anion transport function and seems to have an increased tendency to form oligomers which exhibit increased association with the membrane cytoskeleton (Liu et al, 1995), increasing membrane rigidity and decreasing red cell deformability. The deletion impairs the ability of the first transmembrane segment to integrate into the membrane; however, once integrated it assumes a transmembrane disposition (Cheung and Reithmeier, 2005). SAO is widespread in the Southeast Asia regions including southern Thailand, Malaysia, Indonesia, the Phillipines, and Papua New Guinea (PNG). Its prevalence is about 3% in the southern Thai population (Yenchitsomanus et al, 2003) but higher in Indonesian islands and parts of PNG (Nurse et al, 1992) and it is as high as 35% in the population of the north coast of PNG (Mgone et al, 1996). SAO is found only in the heterozygous state of *SLC4A1* ΔAla400-ΔAla408 deletion, its homozygous state is likely to be lethal (Liu et al, 1994; Mgone et al, 1996), for the fetus carrying homozygous SAO mutation may not survive *in utero*. High frequencies of *SLC4A1* SAO mutation in Southeast Asian populations may result from a selective survival advantage against malaria infection because the patient with SAO is less susceptible to cerebral malaria (Allen et al, 1999).

The heterozygous *SLC4A1* SAO mutation does not cause dRTA (Vasuvattakul et al, 1999; Bruce et al, 2000). The effect of the SAO deletion on stability and trafficking of AE1 and kAE1 was examined in transfected HEK 293 cells and kAE1 in MDCK cells (Cheung et al, 2005). Expression levels and stabilities of SAO proteins were significantly reduced in HEK 293 cells. The mutant AE1 protein was retained intracellularly in these cells without detection at the cell surface. The mutant protein is misfolded but its homodimers and heterodimers with the normal proteins could be formed. While kAE1 was localized to the cell surface or the basolateral membrane after polarization of MDCK cells, kAE1 SAO was retained intracellularly. In co-expression of kAE1 SAO and kAE1 in MDCK cells, kAE1 SAO was partly retained intracellularly and co-localized with kAE1 at the cell surface. Thus, Cheung et al (Cheung et al, 2005) have proposed that in the kidney of heterozygous SAO patients, homodimers of kAE1 and heterodimers of kAE1 SAO and kAE1 traffic to the basolateral membrane of the α-intercalated cells, while homodimers of kAE1 SAO are retained in the endoplasmic reticulum and rapidly degraded. This would result in sufficient expression of kAE1 to maintain adequate bicarbonate reabsorption and proton secretion without the phenotype of dRTA.

However, co-existence of SAO and dRTA results from compound heterozygous *SLC4A1* SAO and another dRTA mutation present on the opposite allele, indicating an autosomal recessive manner of inheritance (Vasuvattakul et al, 1999; Bruce et al, 2000; Wrong et al, 2002; Yenchitsomanus, 2003). Compound heterozygous *SLC4A1* SAO/G701D mutations are frequently observed in pediatric patients in the populations of Thailand and other Southeast Asian countries (Vasuvattakul et al, 1999; Bruce et al, 2000; Wrong et al, 2002; Yenchitsomanus, 2003). Other genotypes occurring from compound heterozygous *SLC4A1* mutations with or without SAO were also noted (Bruce et al, 2000; Sritippayawan et al, 2004). The details of these compound heterozygous *SLC4A1* mutations are discussed in the section of AR dRTA.

Autosomal dominant distal renal tubular acidosis (AD dRTA)

The association between *SLC4A1* mutations and AD dRTA has been well documented (Bruce et al, 1997; Jarolim et al, 1998; Karet et al, 1998; Weber et al, 2000; Sritippayawan et al, 2003). The most common mutation resulting in AD dRTA is a missense substitution of Arg589, a conserved residue located within the region of TM6 and TM7 of AE1 protein, predominantly by histidine (R589H) or cysteine (R589C) and rarely by serine (R589S) (Table 1 and Figure 2). The presence of mutations at the same position in several populations with allelic heterogeneity and at least two *de novo* mutations (Karet et al, 1998; Sritippayawan et al, 2003) makes it unlikely that this frequently observed R589H mutation will result from founder effect but is more likely to result from recurrent mutations. This led to the suggestion that codon 589 (CGC) is a 'mutational hotspot' of *SLC4A1* (Sritippayawan et al, 2003), and the mechanism of recurrent mutations probably involves methylation and deamination that alter cytosine (C) to thymine (T) in the CpG dinucleotides (Wrong et al, 2002; Sritippayawan et al, 2003).

The first three *SLC4A1* mutations to be identified in AD dRTA (R589H, R589C, and S613F) were heterozygous, and had normal red cell morphology and eAE1 content (Bruce et al, 1997). Red cell anion transport activity of the patients with the R589H or R589C mutations were slightly reduced (approximately 80% of normal) while those of the patients with S613F mutation were greatly increased (262% of normal). When expressed in *Xenopus* oocytes, only eAE1 R589H showed decreased (40% of normal) chloride influx activity whereas kAE1 R589H showed normal activity. The kAE1 R589C and S613F mutants did not show a significantly different transport function compared with wild-type kAE1. Co-expression of wild-type

Table 1. *SLC4A1* mutations causing autsomal dominant (AD) and autosomal recessive (AR) distal renal tubular acidosis (dRTA)

Mutations causing AD dRTA	Mutations causing AR dRTA
Band 3 PRIBRAM[a]	SAO (ΔAla400-Ala408)[c]
R589H	V488M (Coimbra)
R589C	R602H (Songkla I)
R589S	G701D (Bangkok I)
G609R	S773P (Siriraj I)
S613F	ΔV850
A858D[b]	
A888L+889X	
R901X (Walton)	

Notes:
[a]Band 3 PRIBRAM is a mutation owning to a *SLC4A1* IVS12+1G>T substitution (see text). Heterozygous band 3 PRIBRAM results in incomplete dRTA.
[b]Heterozygous A858D mutation causes incomplete dRTA; complete dRTA is occurred from its compound heterozygous conditions with other recessive mutations (e.g. A858D/SAO and ΔV850/A858D). Thus, the assignment of band 3 PRIBRAM and A858D mutations under AD dRTA may be questionable.
[c]SAO, Southeast Asian ovalocytosis - an in-frame nine-amino acid deletion (ΔAla400-Ala408), is regarded as a mutation causing AR dRTA because its compound heterozygous conditions with G701D or other recessive mutations results in dRTA with ovalocytic red cells. Homozygous SAO has not been reported, and is believed to be lethal *in utero*.
(Bruce et al, 1997 & 2000; Cheidde et al, 2003; Jarolim et al, 1998; Karet et al, 1998; Ribeiro et al, 2000; Rungroj et al, 2004; Sritippayawan et al, 2003 & 2004; Tanphaichitr et al, 1998; Vasuvattakul et al, 1999; Weber et al, 2000;Yenchitsomanus et al, 2002; Yenchitsomanus, 2003).

and mutant AE1 did not show any effect on the chloride uptake function in *Xenopus* oocyte, suggesting that dominant dRTA does not result from a change in transport activity. The conserved Arg589 is located in the region close to TM6 and TM7, which is important for anion binding activity, and S613F change may distort the conformation of the cytoplasmic loop between TM6 and TM7 which contains the putative anion binding site.

Three further unrelated families with the *SLC4A1* R589H mutation associated with AD dRTA were later reported (Jarolim et al, 1998). The individuals with this mutation had normal red cell morphology with a slight decrease (~20%) in sulfate influx activity. kAE1 R589H expressed in *Xenopus* oocytes showed a 20-50% reduction in Cl^-/Cl^- and Cl^-/HCO_3^- exchange but the anion transport activities between wild-type and mutant eAE1 were not different. No dominant negative behavior on the anion transport activity was observed in the co-expression of wild-type and mutant eAE1 or kAE1 in the oocytes (Jarolim et al, 1998). From screening 26 kindreds with primary dRTA for mutations in *SLC4A1*, Karet et al (Karet et al, 1998) found no mutations in this gene in any of the kindreds with AR dRTA, confirmed by linkage analysis. In contrast, heterozygous mutations in *SLC4A1* were identified in one dominant dRTA kindred, one sporadic case, and one kindred with two affected brothers. In these individuals, *SLC4A1* R589S, *de novo* R589H, or R901X due to an intragenic 13-bp duplication resulting in deletion of the last 11 amino acids of AE1 (band 3 Walton), respectively, were identified, indicating the key role of Arg589 and the C terminus in normal AE1 function. However, the suggestion in this paper that the defects in *SLC4A1* were not responsible for AR dRTA has been proved incorrect by the subsequent studies of AR dRTA associated with *SLC4A1* mutations in Thai and other Southeast Asian populations (Tanphaichitr et al, 1998; Vasuvattakul et al, 1999; Bruce et al 2000; Wrong et al, 2002; Yenchitsomanus et al, 2002; Yenchitsomanus, 2003) (Table 1).

Two novel *SLC4A1* mutations resulting in AD dRTA were recently reported. A heterozygous novel mutation occurred from a 20-bp deletion in exon 20 of *SLC4A1* leading to mutation in codon 888 followed by a premature termination codon at position 889 (A888L+889X), truncating the protein by 23 amino acids, was identified in two affected brothers with dRTA, nephrocalcinosis, and failure to thrive, and in their father who had incomplete dRTA (Cheidde et al, 2003). A novel missense G609R mutation causing AD dRTA was reported in affected members of a large Caucasian pedigree who all exhibited dRTA with prominent nephrocalcinosis and progressive renal impairment (Rungroj et al, 2004) and AD dRTA in this family was not associated with loss of anion transport function of the mutant protein but associated with its mis-targeting which will be discussed later.

The results of these studies clearly indicate that *SLC4A1* mutations can cause AD dRTA. However, the molecular mechanism of AD dRTA was mainly unclear. Although the *SLC4A1* mutations were found to be co-segregated with the disease in the several affected families, the mutant proteins did not have significantly functional change as examined in red cells and *Xenopus* oocytes. This indicated that the defects are not simply a loss of anion transport activity but probably caused by other mechanisms, and that these two cell types might not serve as good systems for studying the defects, because the mutant AE1 proteins could move to the surface of the red cells and oocytes, mediating normal transport activities. It was found in the subsequent studies using transfected cell systems that trafficking defects of mutant kAE1 proteins are the major pathogenic mechanisms.

Autosomal recessive distal renal tubular acidosis (AR dRTA)

The *SLC4A1* mutation associated with AR dRTA was first reported in two Thai sibs (Tanphaichitr et al, 1998) who had hemolytic anemia and abnormal red cells with xerocyte-like dumbbell morphology but normal red cell AE1 content and anion transport activity. They were found to carry a homozygous *SLC4A1* mutation resulting in a substitution of glycine by aspartic acid at position 701 in the AE1 protein (G701D), namely *band 3 Bangkok I*, which also links to two polymorphisms, M31T and K56E. When expressed in *Xenopus* oocyte, both eAE1 and kAE1 G701D showed lack of cell surface expression and anion transport activity. When co-expressed with a red cell AE1 chaperone, glycophorin A (GPA), the plasma membrane expression and chloride influx activity of eAE1 and kAE1 G701D could be rescued. Thus, in patients' red cells which normally contain GPA, eAE1 G701D will have surface expression and function, explaining the normal red cell anion transport activity. The renal phenotype is explained by the absence of GPA expression in the α-intercalated cells. Severe hemolytic anemia and red cell abnormality in the two affected sibs in this family are most likely due to the presence of both homozygous *SLC4A1* G701D mutation and homozygous hemoglobin (Hb) E, because the finding of severe hemolytic anemia were not observed, but only slight red cell changes were noted, in seven patients with homozygous *SLC4A1* G701D mutation without homozygous Hb E from five additional Thai families studied (Yenchitsomanus et al, 2002). The homozygous *SLC4A1* G701D mutation seems to be a frequent cause of AR dRTA in Thai pediatric patients from northeastern Thailand (Yenchitsomanus et al, 2002).

The presence of both *SLC4A1* SAO and G701D mutations in Thai and Southeast Asian populations leads to the possibility of compound heterozygosity of these two mutations in the same individual. Two cases of AR dRTA and SAO resulted from compound heterozygous *SLC4A1* SAO/G701D mutations were originally reported in two Thai families from southern Thailand (Vasuvattakul et al, 1999). These patients were not anemic but had little morphological changes of red cells in addition to SAO, and had a decrease of red cell sulfate flux of about 40%. As hemopglobinopathies (e.g., Hb E), and thalassemias are highly common genetic defects in Thailand and other Southeast Asian countries and as the interaction between homozygous *SLC4A1* G701D mutation and homozygous Hb E appeared as an example in the first Thai family reported (Tanphaichitr et al, 1998), other types of genetic interaction within the same individual would be anticipated. Indeed, two additional families with AR dRTA caused by the compound heterozygous *SLC4A1* SAO/G701D genotype from the central Thailand and coexistence of thalassemias leading to dRTA and anemia are recently observed by our group (Yenchitsomanus et al, data to be published). The presence of Thai families with compound heterozygous *SLC4A1* SAO/G701D mutations most likely occur from a mixture between the northeastern Thai population that has a high frequency of the G701D mutation, and the southern Thai population that has a high frequency of *SLC4A1* SAO (Yenchitsomanus et al, 2003). The southward migration of the *SLC4A1* G701D allele seems to reach as far as Malaysia.

Three patients with AR dRTA with compound heterozygous *SLC4A1* SAO/G701D mutations were later reported in two Malaysian families, and AR dRTA in another patient from a Malaysian family resulted from compound heterozygous *SLC4A1* SAO/A858D mutations (Bruce et al, 2000). In the same study, AR dRTA associated with other genotypes including SAO/ΔV850, ΔV850/ΔV850, and ΔV850/A858D was observed in patients from six Papua New Guinean families. Hemolytic anemia and abnormal red cell properties were noted in these patients. Red cell and *Xenopus* oocyte expression studies showed that the AE1 ΔV850 and A858D mutant proteins have greatly decreased anion transport when present as compound heterozygotes (ΔV850/A858D, SAO/ΔV850 or SAO/A858D) with only 3% of the normal red cell sulfate efflux for SAO/A858D, the lowest anion transport activity reported. Similar to the heterozygous *SLC4A1* mutation causing band 3 PRIBRAM (Rysava et al, 1997), the heterozygous A858D mutation was found to be associated with incomplete dRTA. The complete dRTA was observed in compound heterozygous (ΔV850/A858D or SAO/A858D) genotypes. The mutant AE1 A858D protein seems to possess a greater structural or functional abnormality than other mutant AE1 proteins resulting in AR dRTA. *SLC4A1* A858D has been, however, assigned as a mutation causing AD dRTA by the first reporting group (Bruce et al, 2000). It should be noted that the dominant and recessive diseases are generally defined by clinical phenotypes, which may be variable in expressivity and penetrance and can also be modified by the effect of other genes. On the other hand, abnormal laboratory findings can be observed in the heterozygous carriers of recessive diseases that usually present normal phenotypes.

The presence of the homozygous V488M (band 3 Coimbra) mutation causing severe HS and dRTA in a young Portuguese child (Ribeiro et al, 2000) further confirms the AR form of dRTA associated with *SLC4A1* mutations. Two additional novel *SLC4A1* mutations in 3 patients with AR dRTA from 2 unrelated Thai families were recently reported by our group (Sritippayawan et al, 2004). In the first family, the patient with dRTA, rickets, failure to thrive and nephrocalcinosis, had novel compound heterozygous G701D/S773P mutations. In the second family,

the patient and his sister had dRTA and SAO of different clinical severity. While the patient had rickets, nephrocalcinosis, hypokalemia, metabolic acidosis, and inappropriately high urine pH, his sister was asymptomatic and not acidotic but her urine pH level could not be lowered to below 5.5 after a short acid load i.e. incomplete dRTA. Both siblings had compound heterozygous *SLC4A1* SAO/R602H mutations but the cause of this different clinical severity is unknown. It might be an example of the variable expressivity of dRTA phenotype attributable to a modifier gene. The AE1 S773P and R602H are named *band 3 Siriraj I* and *Songkla I*, respectively (Sritippayawan et al, 2004). The locations of *SLC4A1* mutations causing AR dRTA are also shown in Figure 2.

It is obvious that AD dRTA caused by *SLC4A1* mutations was mainly found in occidental patients whereas AR dRTA associated with *SLC4A1* mutations was frequently detected in oriental patients. The possibility that the *SLC4A1* mutations discovered in Southeast Asian populations might have evolved locally because they provide some protection against the clinical effects of *Plasmodium falciparum* malaria as occurs with the *SLC4A1* mutation that gives rise to SAO has been raised (Wilairat, 2000; Bruce et al, 2000).

MOLECULAR MECHANISMS OF AD dRTA CAUSED BY *SLC4A1* MUTATIONS

The molecular mechanisms of AD dRTA associated with two *SLC4A1* mutations, R589H and R901X (band 3 Walton), have been intensively examined. Quilty et al (Quilty et al, 2002a and 2002b) has demonstrated that dominant negative effect would account for the AD dRTA phenotype. eAE1, eAE1 R589H, and kAE1 were expressed at the cell surface of transfected HEK 293 cells, whereas kAE1 R589H was retained intracellularly as shown by immunofluorescence, cell surface biotinylation, *N*-glycosylation, and anion transport assays (Quilty et al, 2002a). The reduced cell surface expression of the mutant

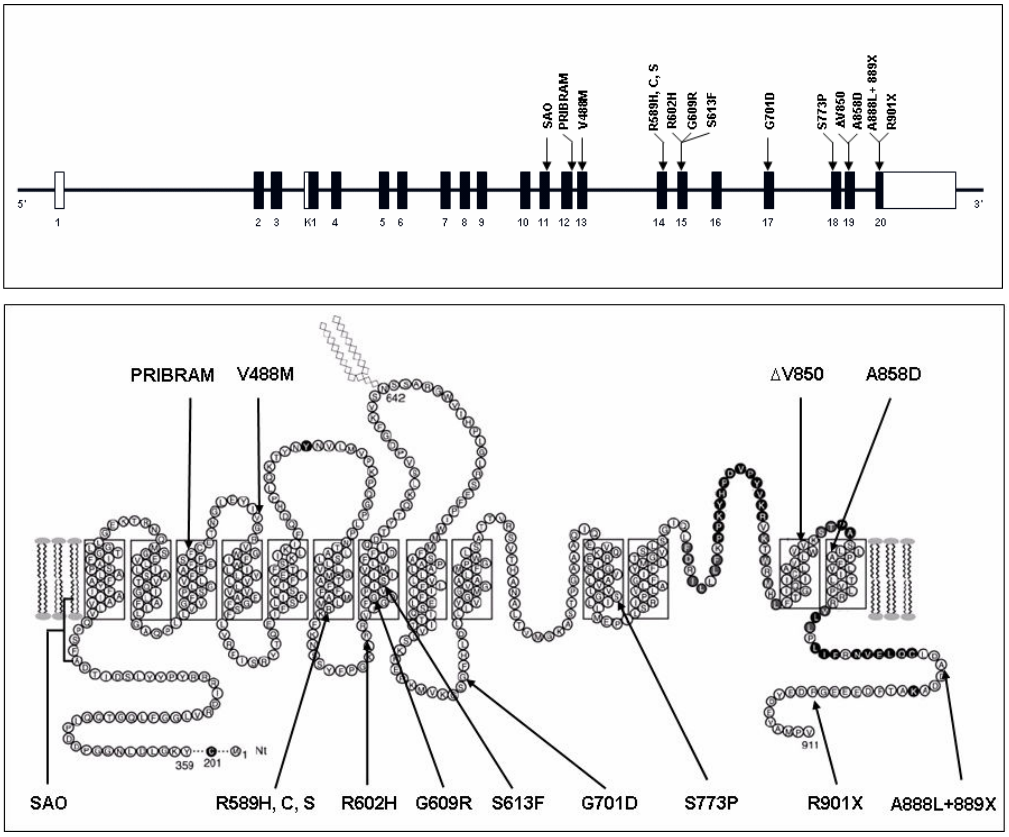

Figure 2. The *SLC4A1* gene and locations of mutations (upper panel). Filled and unfilled vertical bars (or rectangle) represent coding and non-coding exons, respectively. Horizontal lines between the filled and unfilled vertical bars represent introns. Approximate locations of mutations in exons are pointed by arrows and types of amino acid change due to mutations are indicated at the tails of arrows. The structural model of AE1 protein and positions of amino acid alterations (lower panel). Each circle and alphabet represent amino acid in AE1. An incomplete N-terminal domain of AE1 is shown. This AE1 model consists of 13 transmembrane (TM) domains (Zhu et al, 2003). The positions of amino acid change due to mutations are pointed by arrows and types of amino acid alterations are indicated at the tails of arrows. SAO denotes Southeast Asian ovalocytosis. PRIBRAM is AE1 PRIBRAM (an IVS12+1G>T substitution causing an mRNA splicing defect with intron 12 retention and premature termination of protein synthesis), Arg589 can be changed to either histidine (R589H), cysteine (R589C) or serine (R589S).

kAE1 was likely due to its retention in the endoplasmic reticulum (ER). Co-expression of kAE1 R589H reduced the cell surface expression of wild-type kAE1 and eAE1 due to heterodimer formation and a dominant-negative effect. The mutant eAE1 and kAE1 were not grossly misfolded as they could bind to an inhibitor affinity resin. Other *SLC4A1* mutations at the same position (R589C and R589S) also prevented the targeting of kAE1 to the cell surface, indicating that the normal Arg589, which is evolutionary conserved, is important for proper trafficking. In studies using transfected and virally infected MDCK cells originating from principal cells, two groups have similarly observed that the mutant kAE1 R589H and kAE1 S613F retain in ER of the non-polarized cells and also that they distribute throughout the cells with a failure to reach the cell surface in the polarized cells (Toye et al, 2004; Cordat et al, 2005). A co-expression study in MDCK cells showed that the kAE1 R589H similarly retained the wild-type kAE1 intracellularly, probably through heterodimer formation (Cordat et al, 2005).

The properties of kAE1 R901X expressed in *Xenopus* oocytes, non-polarized MDCK, and HEK 293 cells have been examined (Toye et al, 2002; Quilty et al, 2002b). kAE1 R901X had a normal chloride transport activity when expressed in *Xenopus* oocytes. While normal kAE1 was expressed at the cell surface in the cultured kidney cell line, the kAE1 R901X was retained intracellularly within the cultured cells, indicating that the C-terminal tail of AE1, which is truncated in kAE1 R901X, is required for its movement to the cell surface in kidney cells. It was proposed (Toye et al, 2002) but not proved that kAE1 R901X gives rise to dominant dRTA by inhibiting the movement of normal kAE1 to the cell surface, resulting from the association of the normal and mutant proteins in kAE1 hetero-oligomers. It was truly proved by Quilty et al (Quilty et al, 2002b) that this dominant-negative effect was due to hetero-oligomer formation of the mutant and wild-type proteins, because co-expression of the wild-type and mutant kAE1, or eAE1 R901X, indeed resulted in intracellular retention of the wild-type proteins in a pre-medial Golgi compartment in the expression experiments using transiently transfected HEK 293 cells. A series of truncations at the C-terminus of AE1 also resulted in impaired ER exit, depending on the extent of the truncation (Cordat et al, 2003). Intracellular retention of kAE1 in the renal α-intercalated cells by the dRTA mutant would account for the impaired bicarbonate extrusion to interstitium and acid secretion into the urine that is characteristic of dRTA.

The distribution of epitope-tagged full-length wild-type and AE1 R901X was examined in polarized MDCK cells and rat IMCD cells (which express AE1) (Devonald et al, 2003a). In both cell types, the wild-type AE1 localized to the basolateral plasma membrane domain whereas AE1 R901X was found at both the basolateral and apical cell surfaces as well as intracellularly, suggesting that AD dRTA is caused not only by a decrease of AE1 protein at the basolateral surface but also by its inappropriate presence at the apical surface. The apical chloride and bicarbonate flux would alter electrochemical balance across the cell, impairing both unidirectional basolateral bicarbonate extrusion and apical proton secretion. The non-polarized distribution of mutant AE1 could be explained by the loss of a basolateral targeting signal present in the deleted portion of its C terminus. The motif YDEV (904–907) present in the tail of AE1 conforms to a subset of tyrosine-based targeting motifs, YXXØ. When Tyr904 was mutated to alanine (Y904A), the distribution of protein was similar to that of AE1 R901X. YXXØ motifs interact with μ subunits of adaptor-protein complexes, one of which, AP-1B, is specific to polarized epithelial cells. Whether sorting of AE1 depends on AP-1B or not was investigated in the proximal renal tubular cell line, LLC-PK1, which was reported to lack the μ1B subunit and thus cannot form AP-1B complexes. The results suggested that an adaptor protein other than AP-1B is involved in basolateral sorting of AE1 (Devonald et al, 2003a).

The mis-targeting to the apical membrane of kAE1 R901X in stably transfected polarized MDCKI cells was also confirmed in a subsequent study, and it was similarly demonstrated that the 11 C-terminal residues of kAE1 containing a tyrosine-dependent basolateral targeting signal was not recognized by μ1B-containing AP-1 adaptor complexes (Toye et al, 2004). In addition, in the absence of the N-terminus of kAE1, the C-terminus was not sufficient to localize kAE1 to the basolateral membrane, suggesting that a determinant within the kAE1 N-terminus co-operates with the C-terminus for kAE1 basolateral localization (Toye et al, 2004 and 2005).

A missense *SLC4A1* mutation, glycine 609 to arginine (G609R), giving rise to AD dRTA was reported in an extended Caucasian family (Rungroj et al, 2004). The kAE1 G609R expressed on the cell surface of *Xenopus* oocytes maintained normal anion transport function. In contrast to the normal basolateral localization of wild-type kAE1, the mutant kAE1 G609R was partailly located at the apical and sub-apical membrane, and also at the basolateral membrane in polarized MDCK cells, similar to the kAE1 R901X. Gly609 may play a role in kAE1 protein trafficking. Since this residue is close to Arg589 and Ser613 where their missense mutations cause AD dRTA, this region of AE1 may be involved in the protein trafficking or sorting process (Rungroj et al, 2004). The kAE1 G609R has not been examined for a dominant-negative effect, which might explain the dominant dRTA phenotype. In analogy to the kAE1 R901X, kAE1 G609R, which was similarly mis-targeted in the polarized MDCK cells, may also intracellularly retain the wild-type kAE1 protein.

MOLECULAR MECHANISMS OF AR dRTA CAUSED BY *SLC4A1* MUTATIONS

Based on the previous study of kAE1 G701D in *Xenopus* oocyte (Tanphaicitr et al, 1998; Bruce et al, 2000) showing functional mutant protein with defective trafficking to the cell surface, we propose two possible models for the molecular mechanism of AR dRTA caused by *SLC4A1* mutations. Firstly, in the heterozygous state, the mutant kAE1 *per se* is unable to form homo- and heterodimers and so would be unable to traffic to the cell surface. Thus, only the mutant kAE1 is defective, while the wild-type kAE1 is still intact. The wild-type kAE1 would be able to dimerize

and traffic to the cell surface, and sufficiently maintain the normal transport function. And, secondly, in the heterozygous state, the mutant kAE1 might be able to form homo- and heterodimers, but only the homodimer would be defective in trafficking to the cell surface. In the second model, the heterodimer of the mutant and wild-type kAE1 proteins would be able to traffic to the cell surface and would not exhibit the dominant-negative effect, as observed in AD dRTA.

Since a novel compound heterozygous *SLC4A1* G701D/S773P mutation was recently reported by our group in the Thai patient with AR dRTA (Sritippayawan et al, 2004), the S773 and G701D mutations were then used as examples for the study in order to elucidate the molecular mechanism of AR dRTA caused by *SLC4A1* mutations, and to examine the two proposed models. The biosynthesis and trafficking of kAE1 S773P were studied in transiently transfected HEK 293 cells, expressing the mutant alone or in combination with wild-type kAE1 or kAE1 G701D (Kittanakom et al, 2004). It was found that kAE1 S773P was expressed at only a third the level of the wild-type kAE1, had a two-fold decrease in its half-life, and was targeted for degradation by the proteasome. The mutant protein could not be detected at the plasma membrane of transfected HEK 293 cells and showed predominant immunolocalization in the ER of both HEK 293 and LLC-PK1 cells.

The oligosaccharide on a kAE1 S773P *N*-glycosylation mutant (N555) was not processed to the complex form indicating impaired exit from the ER. The kAE1 S773P

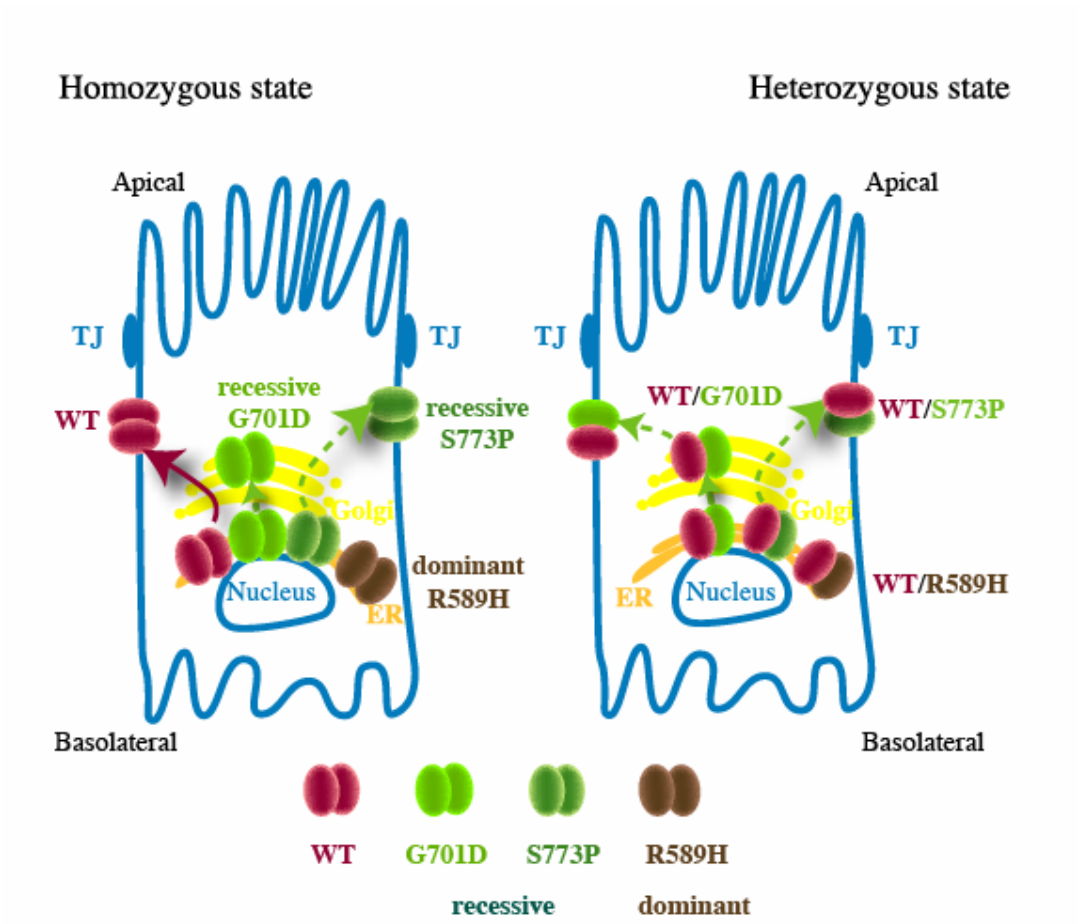

Figure 3. The molecular and cellular model for dominant and recessive dRTA in polarized epithelial cells. Schematic model of epithelial cells expressing wild-type kAE1, dominant or recessive mutants in homozygous (left model) or heterozygous state (right model). Dimers of wild-type kAE1 (burgundy ovals) traffic to the basolateral membrane (solid line) while dominant dRTA mutants (brown ovals) are retained in the ER. Recessive dRTA mutants (green ovals) are partially impaired (dotted lines) in their exit from the ER but can either traffic to the basolateral membrane or are retained in the Golgi apparatus. Heterodimers of wild-type kAE1 and dominant kAE1 mutant are retained in the ER, while heterodimers of wild-type kAE1 and recessive kAE1 mutants can traffic to the basolateral membrane. Theoretically, the proportions of wild-type kAE1 homodimer, heterodimer, and mutant kAE1 homodimer are 25%, 50% and 25%, respectively. Therefore, 25% of kAE1 in the dominant model and 75% of kAE1 in the recessive dRTA model traffic to the plasma membrane. TJ denotes tight junction.

was not properly folded as it showed decreased binding to an inhibitor affinity resin and increased sensitivity to proteases. The kAE1 G701D also exhibited defective trafficking to the plasma membrane. The kAE1 S773P was able to form homodimers and heterodimers with wild-type kAE1 or with kAE1 G701D. Heterodimers of wild-type kAE1 with kAE1 S773P or G701D, in contrast to the dominant mutant kAE1 R589H, were delivered to the plasma membrane (Figure 3). Thus, the second proposed model is likely to be the true one, and the wild-type kAE1 seems to show a 'dominant-positive effect' in rescuing the recessive mutant kAE1 trafficking to the plasma membrane, in contrast with the dominant mutant kAE1 resulting in a 'dominant-negative effect' when heterodimerized with the wild-type kAE1.

In our recent study in MDCK cells (Cordat et al, 2005), we found that the recessive mutants, kAE1 S773P and G701D, showed distinct trafficking defects. The mis-folded recessive mutant kAE1 S773P, while largely retained in the ER in non-polarized MDCK cells, was predominantly targeted to the basolateral membrane in polarized cells while kAE1 G701D was retained in the Golgi in both non-polarized and polarized cells. The co-expression study in MDCK cells confirmed the previous study in HEK 293 cells that the dominant mutant kAE1 R589H retained wild-type kAE1 protein intracellularly while the recessive kAE1 mutants did not. The co-expression of kAE1 S773P and G701D in the same cells showed some co-localization of S773P with G701D in the Golgi, but kAE1 S773P could still traffic to the basolateral membrane. Also, no kAE1 G701D was detected at the cell surface, suggesting that kAE1 S773P did not assist the intracellularly retained kAE1 G701D to traffic to the cell surface as did the wild-type kAE1, despite their ability to oligomerize in the MDCK cells. This result suggests that in the patients with compound heterozygous *SLC4A1* G701D/S773P mutations, only the mis-folded S773P/S773P homodimers which may not properly function can reach the basolateral membrane of the kidney α-intercalated cells, resulting in the development of dRTA.

CONCLUSIONS

The molecular mechanisms of AD and AR dRTA caused by *SLC4A1* mutations have now been elucidated (Figure 3). The modes of inheritance of dRTA associated *SLC4A1* mutations are primarily dictated by the position of amino acid alterations in the encoded kAE1 protein and on some occasions by the type of amino acid replacement, affecting kAE1 folding and molecular structure without significantly changing anion exchange function. The structural alterations of mutated kAE1 in both phenotypic conditions do not generally interfere with its dimerization as homo- and heterodimers. However, they affect intracellular trafficking of the mutant kAE1 homodimers from the ER and trans-Golgi network to the plasma membrane. Between two extremes of normally trafficking of wild-type-kAE1 homodimer and abnormally trafficking of mutant-kAE1 homodimer, trafficking ability of the heterodimer between wild-type and mutant kAE1 in heterozygous conditions exhibits a key role in determining the dominant or recessive nature of the phenotype. In AD dRTA, mutant kAE1 in the heterodimer induces a trafficking defect of wild-type kAE1, so called the 'dominant-negative effect', leading to the AD dRTA phenotype. By contrast, in AR dRTA, wild-type kAE1 in the heterodimer corrects the trafficking defect of mutant kAE1, a newly described 'dominant-positive effect', producing the AR dRTA phenotype. In the case of AD dRTA, the mutant (R589H, S613F, and R901X) homodimers are retained in the ER, as are heterodimers with the wild-type kAE1. The retention of the wild-type protein by the heterodimer formation does not allow sufficient kAE1 to be localized to the basolateral membrane. In some cases (R901X and G609R), the dominant mutants can exit the ER and are partially mis-sorted to the apical membrane, which would also impair the basolateral bicarbonate reabsorption and apical acid secretion of the α-intercalated cells. In the case of AR dRTA, the mutant homodimers can traffic to the Golgi (G701D) or the basolateral membrane (S773P), but since the latter protein is mis-folded, no transport activity is achieved. Heterodimers of AR dRTA mutants can form with the wild-type kAE1, but in this case can traffic to the basolateral membrane. The wild-type homodimers that form can traffic normally to the basolateral membrane. In the heterozygous state, sufficient wild-type kAE1, either as a homodimer or as a heterodimer with an AR dRTA mutant, would be present to maintain sufficient bicarbonate reabsorption and acid secretion.

All these findings may serve as an example in the elucidation of molecular mechanisms of AD and AR phenotypes of the same disease caused by defects of a common gene; to our knowledge this is the first time that the phenotypes of the two different modes of inheritance of the same disease are clearly explained in term of the biochemical properties of the homodimers and heterodimers of the encoded wild-type and mutant proteins.

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STATEMENT OF COMPETING INTERESTS

The authors declared no competing interests.

LIST OF ABBREVIATIONS

AD; Autosomal dominant
AE; Anion exchanger
AP; Activator protein or adaptor protein
AR; Autosomal recessive
CA; Carbonic anhydrase
DIDS; 4, 4'-diisothiocyanostilbene-2-2', disulfonic acid

dRTA; Distal renal tubular acidosis
ER; Endoplasmic reticulum
GPA; Glycophorin A
HS; Hereditary spherocytosis
HEK; Human embryonic kidney
IMCD; Inner medullary collecting duct
LLC-PK1; Renal epithelial cell line from porcine kidneys
MDCK; Madin-Darby canine kidney cells
PNG; Papua New Guinea
SAO; Southeast Asian ovalocytosis
SLC4A1; *Solute carrier family 4, member 1*
TM; Transmembrane
UTR; Untranslated region

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