

PERSPECTIVES IN BASIC SCIENCE

Band 3 mutations, distal renal tubular acidosis, and Southeast Asian ovalocytosis

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Band 3 mutations, distal renal tubular acidosis, and Southeast Asian ovalocytosis. Familial distal renal tubular acidosis (dRTA) and Southeast Asian ovalocytosis (SAO) may coexist in the same patient. Both can originate in mutations of the anion-exchanger 1 gene (*AE1*), which codes for band 3, the bicarbonate/chloride exchanger in both the red cell membrane and the basolateral membrane of the collecting tubule alpha-intercalated cell. Dominant dRTA is usually due to a mutation of the *AE1* gene, which does not alter red cell morphology. SAO is caused by an *AE1* mutation that leads to a nine amino acid deletion of red cell band 3, but by itself does not cause dRTA. Recent gene studies have shown that *AE1* mutations are responsible for autosomal recessive dRTA in several countries in Southeast Asia; these patients may be homozygous for the mutation or be compound heterozygotes of two different *AE1* mutations, one of which is usually the SAO mutation.

Distal renal tubular acidosis and Southeast Asian ovalocytosis have an intimate biochemical relationship that has taken over 30 years to unravel. The story starts with the 1968 description of a family with both diseases [1]. In the original report the red cell abnormality was described as “hereditary elliptocytosis,” though it is now clear that the published blood films showed the ovalocytes of SAO, in keeping with the Filipino origin of the family. The renal disease in this family presented in two sisters under the age of 5 with failure to thrive, hyperchloremic acidosis, hypokalemia, rickets and nephrocalcinosis, and was thus typical of Type 1 or Classic renal tubular acidosis, nowadays often designated “hypokalemic dRTA.” The authors of this original report could not suggest any reason why the two diseases should coexist in the same family, and considered that the associa-

tion had arisen by chance. However, over the years some textbooks have listed “elliptocytosis” among the many causes of secondary dRTA, a suggestion that at one time seemed reasonable, because another red cell abnormality, sickle-cell disease, was found to be associated with defective renal excretion of acid [2], apparently through the effect of sickled red cells in promoting sludging in the renal medullary microcirculation with consequent ischemic renal tubular damage.

Over the years, clinical anecdotes and occasional case-reports [3, 4] have suggested that the association of dRTA and SAO is too frequent to be due to chance, but the confusion between elliptocytosis and SAO has continued to cloud the issue. The distinction between these two similar blood pictures is made clear in Figure 1.

Southeast Asian ovalocytosis is now known to be caused by a mutation of the *AE1* (anion exchanger 1) gene, which codes for band 3, a 911 amino acid protein that is both a structural component of the red cell membrane cytoskeleton and the chloride-bicarbonate anion-exchanger in this membrane (Figs. 2 and 3). Band 3 is the most abundant of red cell membrane proteins, with approximately a million copies of the molecule in each red cell [7–9]. Within the red cell membrane band 3 exists in the form of a dimer or higher oligomer. The mutation causing SAO is a 27-base deletion of the *AE1* gene in chromosome 17 that results in a deletion of the nine band 3 amino acids 400–408 inclusive [10–12]. Invariably this mutation is accompanied by substitution in band 3 of lysine 56 by glutamic acid (K56E), the asymptomatic “Memphis” polymorphism that is common in the general healthy population. The SAO deletion results in a complete loss of anion transport activity in the mutant band 3 [13]. SAO, as its name implies, is found almost exclusively in Southeast Asia (Fig. 4), especially the Malay archipelago, the Philippines, Indonesia and southern Thailand, with a prevalence as high as 30% in the Indonesian island of Sulawesi and parts of Papua New Guinea [14]. The SAO erythrocyte is exceptionally rigid, but the abnormality

Key words: *AE1*, anion exchanger, gene mutation, distal renal tubular acidosis, ovalocytosis, spherocytosis.

Received for publication October 18, 2001
and in revised form February 15, 2002
Accepted for publication February 15, 2002

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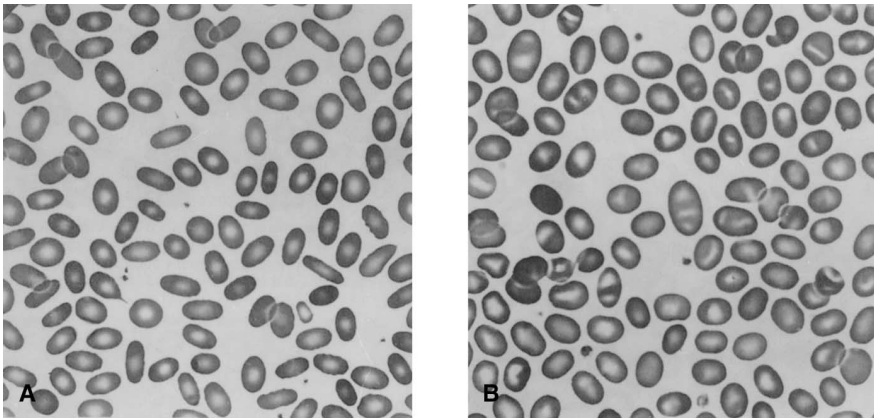


Fig. 1. Blood films in hereditary elliptocytosis (A) and Southeast Asian ovalocytosis (B). In elliptocytosis the long axis of the cells is often more than twice the transverse axis, and there are occasional rod-shaped cells. In SAO the long axis of the oval cells is usually less than twice the transverse axis; many erythrocytes contain a transverse band of pallor (“stomatocytes”) or a well-hemoglobinized ridge, and there are occasional large macrocytes. (Reprinted from *Practical Haematology* by J.V. Dacie and S.M. Lewis, Figures 7.11 and 7.13, pp 101 and 102, 1995 [5] with permission from the publisher, Harcourt Churchill Livingstone.)

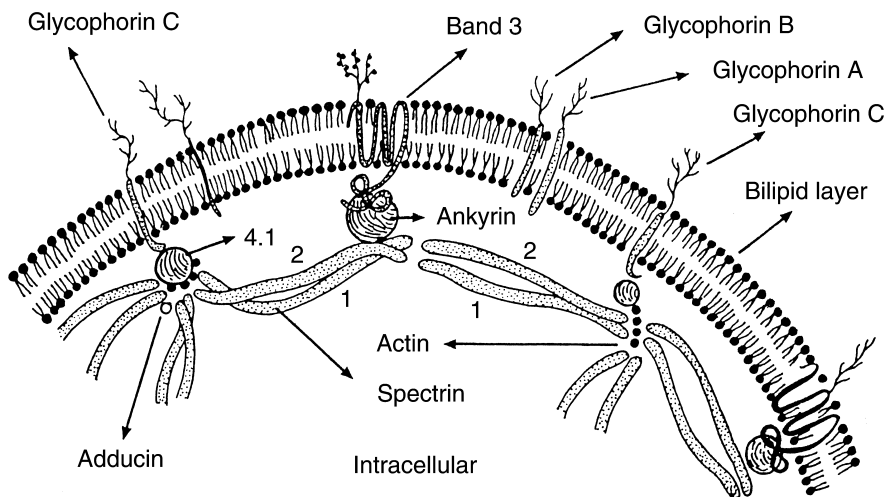


Fig. 2. Structure of erythrocyte membrane. (Reprinted from *The Inherited Metabolic Diseases* by A.J. Grimes and N.C.P. Slater, Figure 14.1, p. 526, 1994 [6], with permission of the publisher Harcourt Churchill Livingstone.)

does not cause hemolytic anemia, and by itself does not give rise to morbidity. SAO is believed to have evolved because these parts of Southeast Asia historically have had a high incidence of *Plasmodium falciparum* malaria, against which SAO offers clinical protection [15, 16]. In this respect SAO resembles sickle-cell disease, the thalassemias, and glucose-6-phosphate dehydrogenase deficiency, all of which are hereditary conditions that are believed to have evolved in areas affected by *P. falciparum* malaria as a result of the protection they provide against this disease. The clinical impression that SAO is more benign than these other diseases is to some extent illusory, for in practice SAO is always in the asymptomatic heterozygous form, the homozygous state apparently being so lethal that it does not survive *in utero*.

PATHOPHYSIOLOGY OF dRTA

The basic fault is generally considered to lie in the proton-secreting alpha-intercalated cell in the renal collecting duct [17]. In this cell the protons to be secreted

are derived, through the enzymatic action of cytoplasmic carbonic anhydrase II, from the hydration of carbon dioxide to carbonic acid, which dissociates to form bicarbonate and hydrogen ions, the latter being secreted into the tubule through the action of an H^+ -ATPase or an H^+/K^+ -ATPase in the luminal (apical) membrane (Fig. 5). The bicarbonate generated by this process leaves the tubular cell by the bicarbonate/chloride exchanger on the basolateral membrane and enters the blood stream.

Endeavors to discover the molecular basis of familial dRTA have been directed at the various component parts of distal proton secretion: the carbonic anhydrase II (CA II) within the tubular cytoplasm, the proton pumps on the luminal membrane (particularly the H^+ -ATPase), and the anion-exchanger on the basolateral membrane. RTA due to CA II mutations is a very rare disease that has more the features of proximal than of distal RTA, in keeping with the wide distribution of CA II throughout the nephron. The nephrocalcinosis of other forms of dRTA is rare in this syndrome, which is characterized by osteopetrosis and cerebral calcification [18]. Of the

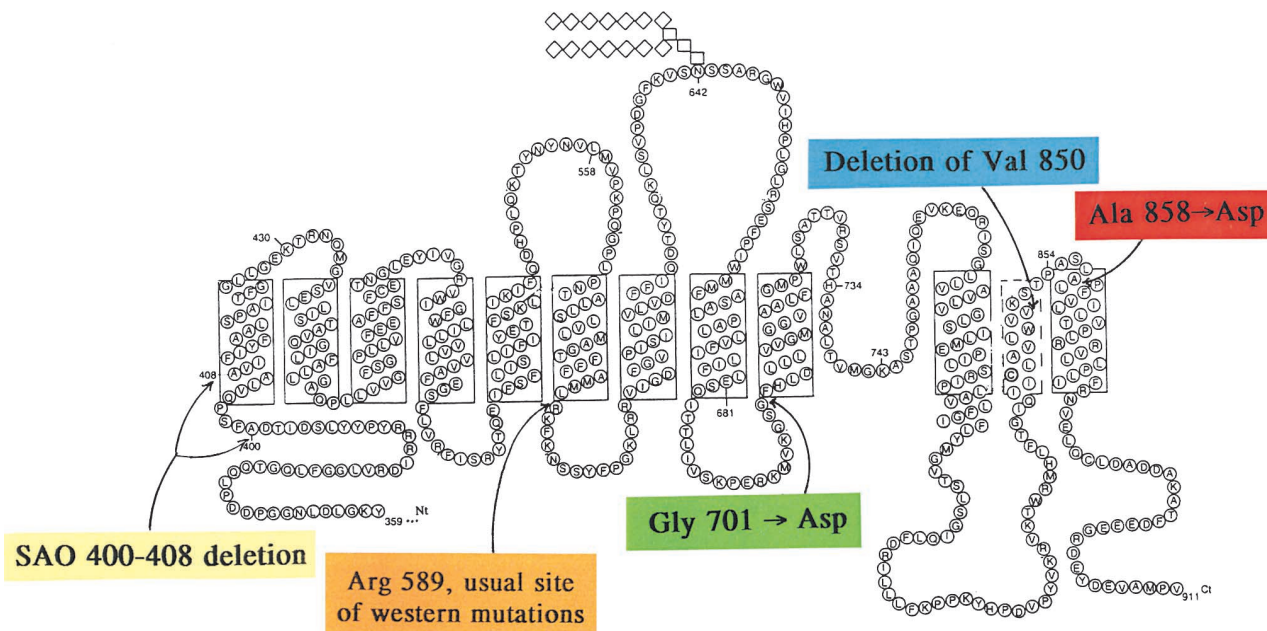


Fig. 3. Band 3, the erythroid bicarbonate/chloride exchanger. The N-terminal 358 amino acids are not shown. Mutations causing SAO and dRTA are shown in color. Redrawn from [9], with permission of the publisher, Taylor and Francis, at <http://www.tandf.co.uk/Journals>.



Fig. 4. Southeast Asia. The area shown covers 5200 miles from west to east and 3400 miles from north to south. Colored arrows represent individual families with dRTA and a band 3 mutation. The PNG family shown with the A858D mutation also had members with the ΔV850 mutation.

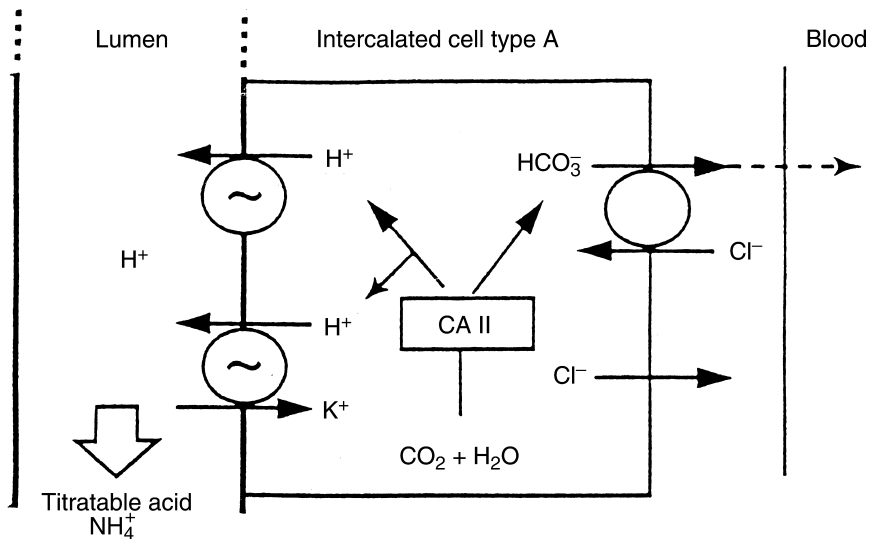


Fig. 5. Ion pathways involved in proton secretion in the renal collecting duct. The bicarbonate/chloride exchanger on the basolateral membrane is the anion-exchanger number 1 (AE1, band 3) truncated at the N-terminal by loss of 65 amino acids. CAII is carbonic anhydrase II. Redrawn from [17], with permission of the authors and the publisher, Lippincott Williams & Wilkins © 2000.

two proton pumps on the luminal membrane, defects of the H⁺/K⁺-ATPase have not yet been incriminated as playing a part in familial dRTA, but recent work has established the critical role of mutations affecting the H⁺-ATPase in autosomal recessive dRTA. The more common form of the disease with sensorineural deafness has been shown to be associated with mutations affecting the ATP6B1 subunit of this H⁺-ATPase, which also is expressed in the cochlea and endolymphatic sac of the inner ear [19], providing a good conceptual framework for the association of this type of dRTA with hearing loss. In the less common form of recessive dRTA with normal hearing, only recently recognized as a separate entity [20], mutations affecting an accessory subunit of the H⁺-ATPase, designated ATP6N1B, have been described recently [21]. In contrast to the recessive disease, dRTA has not yet been established to result from a mutation affecting the H⁺-ATPase in any patient with the dominant form of the disease.

By comparison with the energy-dependent proton pumps on the urinary side of the acid-secreting renal cell, the chloride/bicarbonate exchanger on the opposite side of the cell might seem a less likely candidate for the molecular defect of dRTA. However, this anion exchanger is vital to tubular acid excretion, for loss of its function in the presence of continuing hydrogen-ion secretion by the luminal proton pumps would lead to excessive accumulation of bicarbonate within the cell, with a consequent reduction in the dissociation of carbonic acid and hence reduced availability of protons for secretion into the tubular lumen. The particular relevance of SAO to dRTA lies in the finding that the *AE1* gene codes not only for the band 3 in the red cell, but also for the chloride/bicarbonate exchanger on the basolateral membrane of this renal α -intercalated cell. The

two anion-exchangers are identical except that the renal isoform is truncated of the 65 N-terminal amino acids that are present in the erythrocytic form [22–24]. This observation suggested that *AE1* mutations affecting the transport functions of this ion exchanger might be responsible for both SAO and dRTA, and it was with this hypothesis in mind that a search for band 3 mutations in subjects with hereditary dRTA was started about eight years ago. The first observations were described in 1996 [25], and were rapidly confirmed in several other centers.

BAND 3 MUTATIONS IN FAMILIAL dRTA

In familial dRTA in Western populations (Europe, North America and Australia) SAO has not been reported, and red cells are morphologically normal. Affected family members with the dominant disease were found to have *AE1* gene mutations [20, 25–29]. In nine of the first eleven families studied, the mutation consisted of a substitution of the arginine molecule in position 589 of the band 3 molecule (Fig. 3) usually by histidine, though in other families arginine was replaced by cysteine or serine, and two families had mutations affecting other parts of the band 3 molecule including the mutant band 3^{WALTON} described below with an eleven amino acid deletion at the C-terminus of the protein. The search for band 3 mutations in familial dRTA was extended to the recessive form of the disease, but none of the families initially studied, all of Western or Middle-East stock and with normal red cell morphology, were found to have *AE1* mutations [20]. Many of these families were later shown to have mutations affecting the renal H⁺-ATPase, as already described.

Southeast Asia, owing to the prevalence of SAO, should be a fertile area for research into the association of dRTA

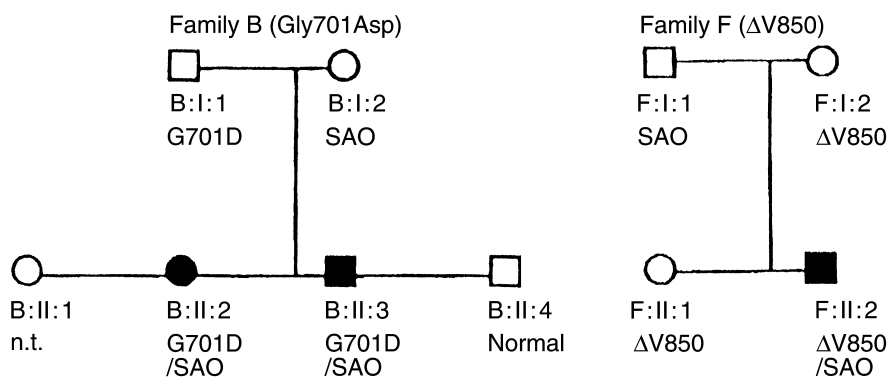


Fig. 6. Family trees of Malaysian family with dRTA, SAO and G701D mutation (left), and PNG family with dRTA, SAO and $\Delta V850$ mutation (right). Squares are males, circles females, solid symbols show dRTA patients, n.t., not tested. Redrawn from [39], with permission from the authors, 2000, *Biochemical Journal*, 350, p. 43, © the Biochemical Society at <http://www.biochemj.org>.

with *AE1* mutations. However, dRTA has not been reported particularly frequently from these regions. The exception has been Thailand. In the northeastern region of this country, where SAO is uncommon, dRTA is sufficiently common to be described as “endemic” [30]. In earlier Thai studies no association between SAO and dRTA was remarked; the prevalence of dRTA was attributed either to chronic dietary deficiency of potassium [31–33], or environmental pollution with vanadium [30, 34], in keeping with in vitro studies showing inhibition of distal nephron H^+/K^+ -ATPase by vanadium [35] and the observation that vanadium toxicity in rats can lead to a state similar to human dRTA [36]. However, more recent genetic studies of dRTA patients in northeast Thailand, who do not have SAO, has revealed six families with missense *AE1* mutations affecting band 3 residue 701, with substitution of glycine by aspartic acid (abstract; Vasu-vattakul et al, *J Am Soc Nephrol* 11:415A, 2000) [37]. This G701D mutation is recessive, with dRTA affecting only positive family members who are homozygous for the defect. In mainland Malaysia and the part of southern Thailand that extends into the Malay peninsula, the identical mutation has been found in a further four dRTA families, but here the G701D mutation runs as a compound heterozygote with the SAO mutation on the other allele [38, 39]. Relatives with either the G701D or the SAO mutation, but not both, were found to acidify their urine normally, as were unrelated subjects with SAO alone. In relation to dRTA, the SAO mutation thus appears to behave as a recessive mutation, causing the renal disease only when accompanied by another band 3 mutation in the opposite allele; homozygous SAO has not yet been reported, but it is likely that such individuals would have dRTA if they survived intrauterine life. Among other defects, these homozygotes would completely lack *AE1*-associated anion transport activity in their red cells and kidneys, and their red cells would be expected to have marked changes in shape and mechanical characteristics which could alter their properties in the circulation.

In Papua New Guinea (PNG), more than 3000 miles

to the east, different band 3 mutations were found to be associated with dRTA in six families in the malarious north coastal areas of the island [39]. Affected family members all had a mutation causing deletion of valine in the 850 position ($\Delta V850$), which was usually present as a compound heterozygote with the SAO deletion, though in one affected child it was homozygous and in another it formed a compound heterozygote with a second mutation involving substitution of aspartic acid for alanine in the 858 position. This latter A858D mutation was described as dominant, unlike the G701D and $\Delta V850$ mutations, for in heterozygous form it was associated with impaired ability to acidify the urine but no acidosis (incomplete syndrome of dRTA) in two families in Malaysia and Papua New Guinea.

Figure 4 shows the location in Southeast Asia of the individual dRTA families so far shown to have band 3 mutations. Figure 6, with two family trees of dRTA/SAO patients, shows how the presence of a compound heterozygote of SAO with a second band 3 mutation (G701D in the Malaysian, and $\Delta V850$ in the PNG family) leads to children with the association of dRTA and SAO. The clinical phenotype of the Malaysian family—two siblings with dRTA/SAO, one parent with SAO and the other apparently healthy—is the same as the Filipino family originally described in 1968 [1], though the identity of the putative second band 3 mutation in that family is unknown.

The few reports available thus far show that the renal phenotype of familial dRTA, whether caused by band 3 mutations or mutations affecting the proton pump subunits ATP6B1 or ATP6N1B, closely corresponds with dRTA as originally established in the West [40], usually presenting in childhood with failure to thrive, systemic acidosis, hypokalemia and nephrocalcinosis, and frequently accompanied by rickets [19–21, 26, 39]. The clinical features vary widely, even within the same family. There is an impression that the dominant disease usually takes a milder form, presenting in late childhood or young adults, whereas the recessive disease commonly presents under the age of four years with the same clini-

Table 1. Recognized genetic varieties of familial distal renal tubular acidosis (dRTA)

Classification of dRTA	Geographical location	Mutation	Clinical presentation	References
Autosomal dominant	Usually Western, probably worldwide	<i>AEI</i> (chromosome 17): R589H, R589C, R589S, S613P, A858D, R901-stop	Late childhood and young adults	[20, 25–29]
Autosomal recessive with sensorineural deafness	Western, Middle East	<i>ATP6B1</i> (chromosome 2): 15 different mutations	Usually infants	[19]
with normal hearing	Middle East	<i>ATP6N1B</i> (chromosome 7): 8 different mutations	Usually infants	[21]
	Southeast Asia	<i>AEI</i> (chromosome 17): G701D, Δ V850; often compound heterozygotes with SAO	Usually infants	

cal features in more severe form (Table 1). However, these apparent differences may result from differences in case selection, for the younger patients with recessive disease have been reported from developing countries where mildly affected and older patients might well escape medical attention. Apart from these possible differences in age of presentation and clinical severity, the only clinical differences between the several genotypes causing the disease are non-renal criteria such as ethnic origin, family history, red cell findings and hearing loss. Familial dRTA thus behaves like some other hereditary forms of renal tubular disease, such as Bartter's syndrome and nephrogenic diabetes insipidus, in that the same renal phenotype may result from more than one genotype.

Hemolytic anemia is not a feature of SAO by itself, or of the dominant band 3 mutations causing dRTA, but is a feature of children who are homozygous for the G701D mutation or have dRTA with the compound heterozygotes G701D/SAO, ΔV850/SAO, A858D/SAO and ΔV850/A858D [39]. Hemolysis has not been reported in recessive dRTA caused by proton pump mutations, but is not to be expected in these syndromes owing to the lack of band 3 abnormalities that would damage red cell integrity.

Band 3 mutations are involved not only in the ovalocytosis of SAO, but also in many cases of hereditary spherocytosis (HS), which unlike SAO is associated with increased osmotic fragility of erythrocytes and hemolytic anemia. The basic fault in HS is a defect in red cell membrane surface area; the condition is genetically heterogeneous and associated with several membrane disorders, including defects in band 3, spectrin and ankyrin. Over 40 different band 3 mutations have been reported in HS, affecting both cytoplasmic and membrane parts of the molecule, the majority of them missense mutations or single amino acid deletions or insertions [41, 42]. With rare exceptions, affected individuals do not usually have the dRTA phenotype. A study of seven unrelated families with HS and band 3 defects showed normal urinary

acidification in six families, but impaired acid excretion (incomplete dRTA syndrome) in two affected members of a seventh family with a heterozygous band 3 mutation (band 3^{PIBRAM}) involving deletion of the C-terminal portion of the molecule including all but the first three transmembrane domains [43]. A catastrophic band 3 mutation has recently been reported from Portugal [44], a valine for methionine 488 substitution (band 3^{COIMBRA}) that in homozygous form led to a barely viable infant with complete absence of erythrocytic band 3, a blood film showing spherocytosis with bizarre red cell elongations, severe hemolytic anemia and complete dRTA, including nephrocalcinosis shown within the first year of life.

DISTRIBUTION OF FAMILIAL dRTA WORLDWIDE

Table 1 summarizes current findings regarding the molecular basis of the main forms of familial dRTA and the geographical and ethnic distribution of the resultant disease. The data are provisional and likely to be radically changed as further discoveries are made. Autosomal dominant dRTA, the result of an *AEI* mutation, is shown as probably worldwide in distribution, and usually the result of a mutation affecting residue 589 in band 3, but except for the A858D mutation found in two families in Southeast Asia, to date only Western families have been studied genetically. Autosomal recessive dRTA caused by H⁺-ATPase mutations has been predominantly the result of intermarriage in Middle Eastern countries, and this is especially so in the disease with intact hearing and *ATP6N1B* mutations, of whom all nine reported families have been from the Middle East, and all but one the product of consanguineous unions [21]. Autosomal recessive dRTA associated with *AEI* mutations has been restricted to families of Southeast Asian origin, in whom it may be the result of a homozygous *AEI* mutation but more often results from a compound heterozygote of SAO with another *AEI* mutation. The countries affected by SAO have a total population of over four hundred mil-

lion, but are relatively undeveloped and medically under-resourced, so the prevalence of this form of dRTA is likely to have been underestimated (Fig. 4). Mutations of the collecting duct H^+/K^+ -ATPase causing dRTA, either dominant or recessive, and mutations of the H^+ -ATPase causing the dominant disease, have not yet been reported.

EVOLUTION OF BAND 3 DEFECTS WITH dRTA

The band 3 mutations demonstrated so far in Southeast Asian dRTA families (G701D, $\Delta V850$ and A858D) occupy different sites in the band 3 molecule from those found in dRTA patients in the West. Furthermore, the G701D and $\Delta V850$ mutations found in Southeast Asia are unlike those found in the West in being recessive rather than dominant. What is the explanation for these differences? In the Western disease nearly all the relevant mutations consist of single amino acid substitutions of arginine 589, usually with histidine but also with cysteine and serine. In two Western families of Irish origin with the histidine substitution, no founder effect could be shown by haplotype analysis [26]. The high frequency of mutations at Arginine 589 to histidine and cysteine most likely results from the recognized propensity of cytosine in CG sequences to undergo mutation to thymine [45]. In Southeast Asia the principal mutations reported to date in dRTA (SAO $\Delta 400-408$, G701D and $\Delta V850$) are recessive in relation to dRTA. There is evidence of a founder effect with all three mutations: In SAO the K56E Memphis polymorphism is invariably found, suggesting a common origin to both mutations in the past. Likewise, the G701D mutation is accompanied by a threonine for methionine substitution in the 31 position, M31T [37, 39]. This latter mutation is unlikely to be related to the renal disease as it is not expressed in the renal isoform, but suggests a founder effect, which would be in keeping with the continuity of the land mass where these mutations have been found (Fig. 4). Finally, in the case of the $\Delta V850$ mutation, the close geographical clustering of affected kindreds to the north coast of Papua New Guinea is suggestive of a founder effect.

The SAO, G701D and $\Delta V850$ mutations carry no morbidity in their heterozygous form, and the suggestion of a founder effect to each mutation implies that the relevant heterozygotes have had some survival advantage in past generations, leading to the wider distribution of the mutation. In the case of SAO this survival advantage is protection against clinical effects of *P. falciparum* malaria, as already mentioned. Parasitized normal erythrocytes become unusually sticky, leading to the occlusion of capillaries that characterizes cerebral malaria. The suggested mechanism for protection in SAO individuals is that this stickiness ("cytoadherence") is reduced by the

abnormal erythrocyte membrane of SAO [15]. No data are currently available to suggest whether the G701D and $\Delta V850$ mutations have this effect, and this is a possible subject for future study.

MOLECULAR BASIS OF RECESSIVE dRTA WITH BAND 3 MUTATIONS

Recessive dRTA appears to result from the absence or a very marked deficiency of chloride-bicarbonate exchange activity in the basolateral membrane of the kidney intercalated cell. In the case of the G701D mutation this occurs because the mutant protein is totally dependent on the presence of glycophorin A (GPA) for its movement to the cell surface. GPA is a glycosylated protein that is associated with band 3 and has a single span across the erythrocyte membrane (Fig. 2). Expression in *Xenopus* oocytes [37, 39, 46] demonstrated that GPA completely rescues the cell surface movement of the G701D mutant band 3 to normal levels. This contrasts with normal band 3, which moves to the cell surface even in the absence of GPA, although GPA further enhances this movement [47]. Red cells contain GPA but GPA is absent from the kidney, hence individuals homozygous for the G701D mutation have normal levels of band 3 in their red cells [37, 39]. It is proposed that, in homozygotes, the mutant G701D protein does not reach the basolateral membrane of the intercalated cell, but is turned over within the cell [37, 39, 46]. In SAO/G701D compound heterozygotes, the SAO protein is presumed to reach the cell surface, but since it is inactive in anion transport [13], it acts as if it were a band 3 null allele. The recessive $\Delta V850$ mutation behaves differently; expression studies in *Xenopus* oocytes show that it is expressed very poorly at the cell surface in the absence of GPA, and GPA only partially rescues the cell surface movement of the $\Delta V850$ mutant [39]. In this case the disease probably results from inadequate anion exchange activity in renal tubular intercalated cells as a result of a much decreased level of band 3 [39].

The presence of the SAO mutation clearly has a deleterious effect on the function of the normal allele, or other mutant allele in compound heterozygotes. These effects show most clearly in the altered hematology and red cell properties in these individuals. It has already been noted that the red cells of simple SAO heterozygotes are more rigid than normal, although these individuals do not have anemia. In addition, the specific anion transport activity per band 3 molecule of the normal protein in SAO red cells is reduced to 75% of that in normal red cells [13, 39]. Even more striking changes occur in the red cells of individuals who are compound heterozygotes of SAO and dRTA mutations [39]. Compound heterozygotes of SAO with the G701D and A858D mutations had red cell membrane rigidity

that was markedly higher than even the simple SAO heterozygotes in the same families. The specific anion transport activity per band 3 molecule in the red cells of compound heterozygotes of SAO and the A858D or Δ V850 mutations was reduced to 17% and 55%, respectively, of the activity of the normal protein, although the mutant proteins had much higher activity in simple heterozygotes. Finally, compound heterozygotes of SAO with the G701D, Δ V850 or A858D mutations all showed anemia of variable degree, but there was no evidence of this in simple heterozygotes of these dRTA mutations in the same families [39]. These effects clearly demonstrate that the band 3 SAO mutation alters the structure of these dRTA mutant band 3 alleles, usually in a detrimental fashion. These changes in properties very likely occur within band 3 hetero-oligomers, as a result of interactions between the SAO protein and the dRTA mutant protein that alter the structural and functional characteristics of the dRTA protein. Evidence for interactions of this type also comes from co-expression studies in *Xenopus* oocytes [39]. Similar interactions with the SAO protein almost certainly occur in the kidney and are likely to modify the functional properties of the dRTA mutant proteins in intercalated cells.

MOLECULAR BASIS OF DOMINANT dRTA WITH BAND 3 MUTATIONS

The usual band 3 mutation in this form of RTA is a single amino acid substitution of arginine 589. Here dRTA clearly does not result simply from a reduction in the anion exchanger activity in the kidney, since the presence of the anion transport-inactive SAO allele or the other transport-inactive mutant alleles found in hereditary spherocytosis (discussed below) that can decrease red cell anion exchange by more than 50%, are not normally associated with dRTA. In addition, most of the dominant dRTA band 3 mutant proteins have almost normal anion transport activity [26]. Several possible mechanisms for dominant dRTA have been proposed [26]: interactions between the normal and mutant proteins in band 3 oligomers that alter the targeting properties of the normal protein, either by preventing the movement of the normal and mutant proteins to the cell surface or sending them to an inappropriate membrane; mistargeting of the mutant protein to the apical membrane of the intercalated cell, thus neutralizing the proton gradient induced by the H^+ -ATPase by allowing the exit of bicarbonate with the protons pumped into the urinary fluid; or the mutant band 3 may form a membrane channel or leak in the plasma membrane of the intercalated cells that would dissipate the ionic gradients across the cell that are required for the net secretion of acid. Different dominant dRTA mutations may well act in different ways and ex-

perimental verification of the mechanisms involved will be needed for each of the mutants.

Recent studies of the expression of a band 3 mutant (band 3^{WALTON}) expressed in red cells and renal tubular cells suggest a mechanism for the dominant dRTA in this case [48]. In band 3^{WALTON} the eleven C-terminal amino acids 901-911 are deleted. The mutant protein is expressed in red cells but at a reduced level, and transport studies of the red cells and the protein expressed in *Xenopus* oocytes have shown normal anion transport activity. In contrast, although normal kidney band 3 was expressed at the cell surface in a kidney cell line, the Walton kidney band 3 was retained within the cytoplasm of these cells and did not migrate to the cell membrane. Band 3^{WALTON} is clearly targeted differently in erythrocytes and kidney cells, and the presence of the normal band 3 C-terminal tail is required for movement to the cell surface of kidney cells. It is likely that dominant dRTA arises because the mutant protein inhibits the movement of normal band 3 to the cell surface. Thus, the association of the normal and mutant protein in band 3 hetero-oligomers causes the intracellular retention of the normal band 3 along with the abnormal protein.

The above mechanism also provides an explanation for the puzzling observation that band 3 mutations that cause dominant hereditary spherocytosis (HS) almost invariably do not cause dominant dRTA, and dominant dRTA band 3 mutations do not give rise to HS. Band 3 mutations causing HS lead to a significant decrease in the amount of erythrocyte band 3, which destabilizes and reduces the area of the red cell membrane. In dominant dRTA the mutant proteins are present at substantially normal levels in the affected red cells, and therefore HS does not result. Most dominant *AE1* mutations causing HS either result in an unstable mRNA (and therefore no protein is synthesized) or the protein is misfolded and not incorporated into the erythrocyte membrane [41]. The latter HS mutations are probably recognized and eliminated by the endoplasmic reticulum-associated degradation (ERAD) and quality control system [49] before they have an opportunity to form hetero-oligomers that result in the intracellular retention or mistargeting of normal band 3, which is required to cause dominant dRTA. The rarity of dRTA associated with HS suggests that the association of normal and mutant band 3 proteins in hetero-oligomers may be a general requirement for the manifestation of dominant dRTA.

BAND 3 IN NON-FAMILIAL dRTA

This review is primarily concerned with familial dRTA, but the band 3 bicarbonate/chloride exchanger also may be involved in autoimmune dRTA, the other common form of primary dRTA, which is clinically associated with extrarenal autoimmune disease, particularly

Sjögren's syndrome, polyarthritis, thyroid and liver disease. The phenotype of the two forms of dRTA is very similar, except that autoimmune dRTA is almost exclusively a disease of sexually mature females, and hypokalemia is more common and more profound than in familial dRTA [50]. As in the case of familial dRTA, research has been mainly directed at establishing whether the collecting duct H⁺-ATPase is defective. Renal biopsy material from five such patients, four with Sjögren's syndrome, has shown absent reactivity by monoclonal antibodies to H⁺-ATPase in collecting duct α -intercalated cells in all five patients [25, 51–54], indicating loss of this proton pump. Reports do not make clear whether band 3 was studied by immunohistology in all these patients, but in at least two this was evaluated and absence of the band 3 bicarbonate/chloride exchanger from the same intercalated cells was demonstrated [52, 53], although the intercalated cells were intact and immune staining of band 3 in erythrocytes was normal. This suggests that a selective defect in the tubular anion-exchanger plays a role in causation of this form of dRTA.

ACKNOWLEDGMENTS

This work was supported by grants from the National Kidney Research Fund and the Wellcome Trust. We also thank Dr. R.C. Morris, Jr. and Church & Dwight Co. (Princeton, NJ, USA) and the St. Peter's Trust (University College, London) for financial help with travel, supplies, and the cost of color illustrations. The major part of this work was presented as a poster and was awarded first prize at the 50th Anniversary Meeting of the Renal Association (Cambridge, UK) in September 2000.

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