Q J Med 2012; **105**:861–877
doi:10.1093/qjmed/hcs139

Tropical distal renal tubular acidosis: clinical and epidemiological studies in 78 patients

S. KHOSITSETH[1], L.J. BRUCE[2], S.B. WALSH[3], W.M. BAWAZIR[2,4], G.D. OGLE[5], R.J. UNWIN[3], M.-K. THONG[6], R. SINHA[7], K.E. CHOO[8], W. CHARTAPISAK[9], P. KINGWATANAKUL[10], A. SUMBOONNANONDA[11], S. VASUVATTAKUL[12], P. YENCHITSOMANUS[13] and O. WRONG[3,†]

From the [1]Department of Pediatrics, Faculty of Medicine, Thammasat University, Klongluang, Pathumthani, Bangkok 12120, Thailand, [2]Bristol Institute for Transfusion Sciences, NHS Blood and Transplant, Bristol BS34 7QH, UK, [3]UCL Centre for Nephrology, University College London, London NW2 2PF, UK, [4]Department of Biochemistry, University of Bristol, Bristol BS8 1TD, UK, [5]HOPE worldwide (PNG), Port Moresby, Papua New Guinea, [6]Genetics and Metabolism Unit, Department of Paediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia, [7]Department of Child Health Unit II and Pediatric Nephrology, Christian Medical College and Hospital, Vellore, India, [8]Department of Paediatrics, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, 16150, Kelantan, Malaysia, [9]Department of Pediatrics, Faculty of Medicine, Chiangmai University, Maung district, Chiangmai 50200, [10]Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Pathumvan, Bangkok 10330, [11]Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi, Bangkok10700, [12]Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi, Bangkok10700 and [13]Division of Molecular Medicine, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi, Bangkok10700, Thailand

Address correspondence to R.J. Unwin, UCL Centre for Nephrology, University College Medical School, Royal Free Campus and Hospital, Rowland Hill Street, London NW3 2PF, UK. email: robert.unwin@ucl.ac.uk
[†]The senior author Professor Oliver Wrong died 24 February 2012 while completing this paper.

Received 6 May 2012 and in revised form 1 July 2012

Summary

Background: Distal renal tubular acidosis (dRTA) caused by mutations of the *SLC4A1* gene encoding the erythroid and kidney isoforms of anion exchanger 1 (AE1 or band 3) has a high prevalence in some tropical countries, particularly Thailand, Malaysia, the Philippines and Papua New Guinea (PNG). Here the disease is almost invariably recessive and can result from either homozygous or compound heterozygous *SLC4A1* mutations.

Methods: We have collected and reviewed our own and published data on tropical dRTA to provide a comprehensive series of clinical and epidemiological studies in 78 patients.

Results: Eight responsible *SLC4A1* mutations have been described so far, four of them affecting multiple unrelated families. With the exception of the mutation causing South-East Asian ovalocytosis (SAO), none of these mutations has been reported outside the tropics, where dRTA caused by *SLC4A1* mutations is much rarer and almost always dominant, resulting from mutations that are quite different from those found in the tropics. *SLC4A1* mutations, including those causing dRTA, may cause morphological red cell changes, often with excess haemolysis. In dRTA, these red cell changes are usually clinically recessive and not present in heterozygotes. The high tropical

prevalence of dRTA caused by *SLC4A1* mutations is currently unexplained.
Conclusions: A hypothesis suggesting that changes in red cell metabolism caused by these mutations might protect against malaria is put forward to explain the phenomenon, and a possible mechanism for this effect is proposed.

Introduction

In recent years, it has become clear that familial distal renal tubular acidosis (dRTA) in the tropics differs fundamentally from the disease found in temperate climates. It is almost invariably recessive, it is not uncommon and it is caused by mutations of the *SLC4A1* gene that have never been described outside the tropics. The red cell membrane protein solute carrier family 4 member 1 (SLC4A1; also known as 'band 3' or AE1) is the most abundant protein in the red cell membrane, with approximately 1 million molecules per erythrocyte. This protein consists of a chain of 911 amino acids,[1] of which amino acids 1–399 comprise the N-terminal cytoplasmic portion of the molecule that binds to spectrin by ankyrin in the red cell cytoskeleton and amino acids 400–911 (Figure 1) comprise the C-terminal portion, which includes 12–14 membrane-spanning helices and is responsible for chloride/bicarbonate exchange across the red cell membrane. AE1 is also present on the basolateral membrane of the alpha-intercalated cell of the renal collecting duct, where it is truncated of its 65 N-terminal amino acids and plays an important part in renal acid excretion by facilitating the return to blood of the bicarbonate generated by renal tubular proton secretion.

Mutations of AE1, in either its cytoplasmic or membrane portion, may cause changes in red cell morphology (e.g. spherocytosis, ovalocytosis and stomatocytosis), often with anaemia caused by excess haemolysis. Mutations of AE1 may also reduce AE1 expression or transport activity in the kidney and by interfering with proton secretion by the renal collecting duct, give rise to dRTA, a clinical disorder characterized by defective urinary acid excretion, hyperchloraemic acidosis, renal potassium loss, rickets, renal stones and nephrocalcinosis.

The role of *SLC4A1* mutations in causing dRTA was first recognized 16 years ago,[2,3] and it soon become clear that the inheritance and prevalence of the disease differ markedly in different parts of the world. In temperate countries, dRTA caused by *SLC4A1* mutations is rare and almost invariably autosomal dominant, caused usually by single amino acid substitutions of residue 589 of AE1, less often by single amino acid substitutions of residues 609 and 613 and deletions and insertions affecting the C-terminal of AE1. Red cell morphology is usually unaffected by these mutations, and haemolytic anaemia is not a feature.

In the tropics, dRTA caused by *SLC4A1* mutations is much more common and has a different pattern of inheritance. Only one patient with dRTA caused by a dominant *SLC4A1* mutation has been described: a Thai child in whom the disease resulted from a *de novo* missense mutation of AE1 residue 589,[4] a mutation already described in non-tropical dRTA families. In all other cases, dRTA has been autosomal recessive, caused either by homozygosity of the responsible mutation or compound heterozygosity involving two different *SLC4A1* mutations.[5–15] The mutations involved are different from those found in non-tropical countries: the four most common, and present in numerous unrelated families, are a deletion of AE1 residues 400–408 (which causes a morphological red cell abnormality known as Southeast Asian ovalocytosis [SAO]), missense mutations with amino acid substitutions affecting residues 701 and 858 and a deletion of residue 850, although several other mutations have been described in single families. Typically, these mutations, when present in homozygous or compound heterozygous form, cause morphological red cell changes, often with mild-to-moderate haemolytic anaemia.[16]

Tropical recessive dRTA caused by *SLC4A1* mutations is now one of the commonest forms of familial dRTA reported, with a prevalence that is probably greater than that of familial dRTA in any other population; the reasons for this high local prevalence are unknown. This review describes the clinical phenotype of recessive dRTA caused by these mutations and examines the evidence that the disease has an especially high prevalence in the tropics, and how this might have developed.

Clinical observations

The clinical details of tropical dRTA patients are summarized in Tables 1–5, which includes a total of 78 dRTA patients from 63 families; 59 of these patients have previously been reported and 19 are reported here for the first time. A total of eight different *SLC4A1* mutations gave rise to the dRTA described in these tables. Four of these mutations affected multiple unrelated families: the mutation (deletion of amino acids 400–408) that causes

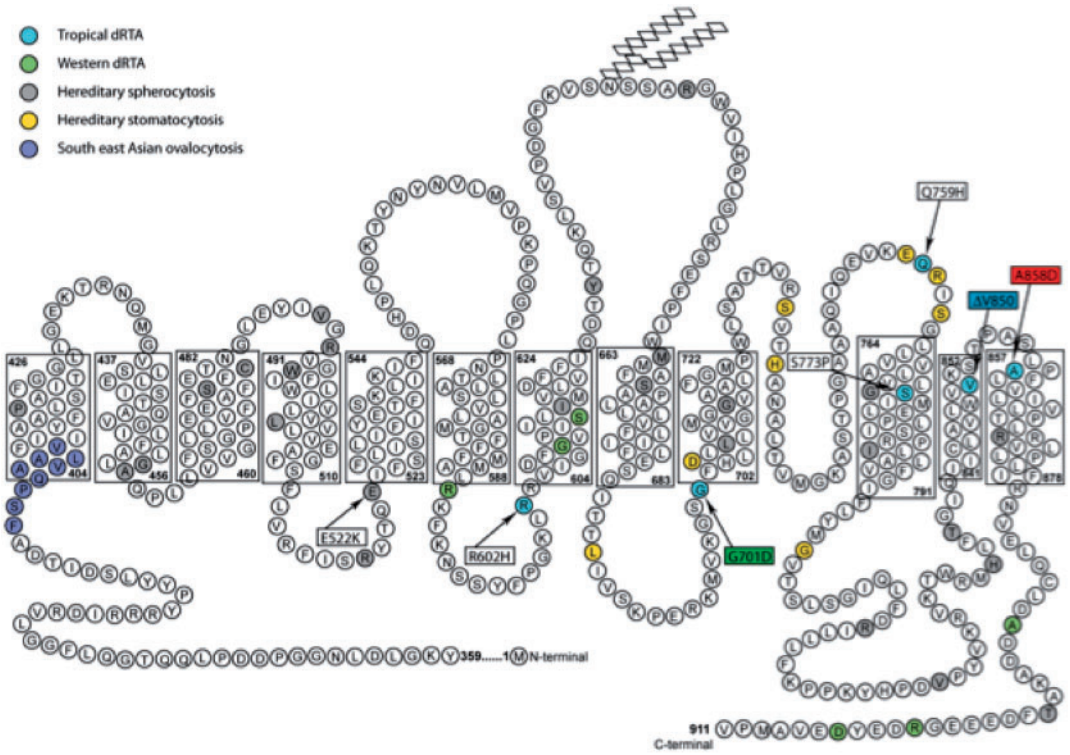

Figure 1. Diagram (adapted[18]) of membrane domain of AE1 showing the position of each reported mutation. The amino acids associated with tropical dRTA are highlighted in pale blue and the nine amino acids deleted in SAO are highlighted in mauve. The dRTA mutations G701D, ΔV850 and A858D are labelled with boxes coloured dark green, dark blue and red to correspond to the labelling on the map shown in Figure 4. Other tropical dRTA mutations are labelled with white boxes. Amino acids associated with western dRTA are highlighted in pale green, those associated with hereditary stomatocytosis are highlighted in yellow and those associated with hereditary spherocytosis in grey.

SAO and the mutations G701D, A858D and ΔV850 shown in Figure 1. These mutations were found in dRTA patients in either homozygous form or as compound heterozygotes with a different *SLC4A1* mutation on the opposite allele. Four other mutations (E522K, R602H, Q759H and S773P) have been found in single families and so far always as compound heterozygotes with a different *SLC4A1* mutation.

The majority of the patients described here were young children, average age 4 years at clinical presentation, but including many infants aged less than a year. Males and females were approximately equally represented: 37 males, 40 females and one patient of non-ascribed gender. At presentation, the most conspicuous feature of these patients was failure to thrive, with body weights usually less than the third centile. Rickets was present in 74% of patients, often with gross skeletal deformities (Figure 2). Medullary nephrocalcinosis was found in 80% of patients in whom it was sought, though probably under-reported, owing to difficulties in organizing renal imaging for some patients; only 12 patients had negative renal imaging for renal calcification, whereas nephrocalcinosis was demonstrated in 52.

On initial presentation, patients had the characteristic blood and urine biochemistry of dRTA, with hyperchloraemic acidosis (mean plasma bicarbonate 12, chloride 115 mmol/l; Tables 1–5) and urine pH values (not shown) that were inappropriately alkaline (pH > 5.5 and usually >6.5) in the presence of acidosis. Hypokalaemia (mean potassium 2.8 mmol/l; Tables 1–5) was present in the majority, particularly in Papua New Guinea (PNG) patients with the ΔV850 mutation, as described later. Plasma creatinine values were usually in the normal range, indicating the absence of significant renal impairment; in a few patients, values were subnormal (e.g. patients 64, 69 and 72; Tables 3 and 4), probably owing to a reduced muscle mass. Some biochemical values are missing for technical reasons or because the original results showing the characteristic changes of dRTA have been mislaid.

Table 6 provides a comparison of these children with those in previously reported families with dominant dRTA caused by *SLC4A1* mutations and recessive dRTA caused by mutations in *ATP6V0A4* or *ATP6V1B1* affecting the renal collecting duct H^+-ATPase.[24–28] This table makes clear that the clinical features of recessive dRTA caused by *SLC4A1*

Table 1 G701D homozygotes associated with dRTA

SLC4A1 genotype	Patient No.	References	Ref. Case No.	Home	Sex/Age	Family history	HCO_3, mmol/l	Cl, mmol/l	K, mmol/l	Creat µmol/l	Rickets	NC	Haemoglobin	
													g/100 ml	Type
G701D/G701D	1	13	II:2	NEThai	M/3.5	Siblings	15	111	2.1	44	+	+	11	E/E
M31T/M31T K56E/K56E	2	13	II:3	NEThai	F/1.0		15	114	2.6	44	+	+	8.3	A/E
	3	15	A:II:1	NEThai	M/1.0	Siblings, PCS	10	118	2.3	27	+	–	12.2	A/A2
	4	15	A:II:2	NEThai	M/2.5		9	119	3.5	44	+	–	12.2	NA
	5	15	B:II:1	NEThai	M/5	Siblings	15	119	2.9	27	+	NA	NA	NA
	6	15	B:II:2	NEThai	M/2.0		21	119	3.8	27	+	NA	NA	NA
	7	15	C:II:1	NEThai	F/1.0		11	121	1.5	62	+	+	11.7	A/E
	8	15	D:II:1	NEThai	F/3.0		14	114	3.6	53	+	+	11.6	A/A2
	9	15	E:II:1	NEThai	F/4		11	113	3.0	44	+	+	NA	NA
	10	10,16	A:II:1	NEThai	M/1.5		16	120	3.1	53	+	+	12.9	12.9
	11	10,16	B:II:1	NEThai	M/3.5		10	123	3.0	44	+	+	14.7	A/A2
	12	10,16	C:II:1	NThai	M/2.5		9	121	2.2	35	+	+	11.7	A/A2
	13	10,16	E:II:1	NThai	M/3.5		7	116	3.2	27	+	–	12.9	A/E
	14	10,16	F:II:1	NThai	M/9		14	118	3.8	44	–	+	12.3	A/E
	15	6	3	Phil	F/4		11	113	2.4	79	+	+	11.0	NA
	16	6	5a	Phil	F/5	Siblings, PCS	15	119	2.8	82	+	+	12.0	A/A2
	17	6	5b	Phil	M/4		9	113	3.4	67	+	+	NA	NA
	18		New	NThai	F2.0	FH	13	106	2.9	53	+	–	NA	A/A2
	19		New	NThai	F/1.0		12	119	4.1	44	–	+	NA	A/A2
	20		New	NEThai	F/4		9	119	3.1	53	–	+	12.6	A/A2
	21		New	NEThai	M/3		10	116	2.7	53	+	+	12	A/A2
	22		New	NEThai	M/3		10	119	3.9	53	+	–	14	A/A2
	23		New	NEThai	F/10		12	123	2.7	53	+	+	14.1	A/A2
	24		New	NThai	M/4		10	116	2.7	53	+	+	12.9	A/A2
	25		New	NEThai	F/1.0		13	100	2.0	35	–	NA	NA	A/E

NEThai, Northeast Thailand; NThai, North Thailand; Phil, Philippines; PCS, parental consanguity; FH, positive family history; NC, nephrocalcinosis; NA, not available.

Table 2 G701D compound heterozygotes associated with dRTA

SLC4A1 Genotype	Patient No.	References	Ref. Case No.	Home	Sex/Age	Family history	HCO_3, mmol/l	Cl, mmol/l	K, mmol/l	Creat, µmol/l	Rickets	NC	Haemoglobin	
													g/100 ml	Type
G701D/SAO	26	14	YAT	SThai	F/<3		14	111	3.4	53	NA	+	NA	A/A2
	27	14	KSN	SThai	M/<3		9	116	3.3	44	NA	+	NA	A/A2
	28	7	A:II:3	Mal	F/4		6	NA	2.2	10	+	+	5.2	NA
	29	7	B:II:2	Mal	F/0.9	Siblings	14	107	3.3	33	+	+	9.8	NA
	30	7	B:II:3	Mal	M/0.6		8	NA	2.9	45	−	+	10.4	NA
	31	5	1	Sarawak	M/5		12	117	2.4	53	−	+	9.0	A/A2
	32	10,16	H:II:1	NEThai	M/1.5		8	119	3.2	80	+	+	12	A/A2
	33	10,16	I:II:1	NThai	M/1.8		14	111	3.3	44	−	+	NA	A/A2
	34	10,16	J:II:2	CThai	M/9		13	115	2.5	53	−	+	8.6	A/A2-α3.7/αα
	35	6	1	Phil	F/0.5		NA	NA	NA	NA	+	+	NA	NA
	36	6	2a	Phil	F/3	Siblings	10	120	2.4	75	+	+	11.0	NA
	37	6	2b	Phil	M/6		13	116	2.3	80	+	+	10.8	NA
	38	6	4	Phil	F/4		9	110	3.5	37	+	+	8.8	NA
	39	6	6	Phil	F/5		13	117	2.8	31	+	−	6.4	NA
	40	6	7	Phil	F/6		13	118	2.7	55	+	+	10.0	NA
	41		New	Mal	M/2		NA	NA	NA	NA	+	+	6.4	NA
	42		New	Mal	M/1.5		9	NA	1.2	NA	+	−	NA	NA
	43		New	SThai	F/8		12	111	3.2	53	+	+	NA	A/A2
G701D/E522K	44	8	1	Taiwan	M/0.6		5	125	3.2	53	−	−	4.6	NA
G701D/S773P	45	12	1	NEThai	M/5		19	96	2.2	53	+	+	13.8	NA
G701D/A858D	46	10,16	K:II:1	SThai	F/5	Siblings	12	114	3.1	53	−	+	13.6	A/E
	47	10,16	K:II:2	SThai	F/5		15	112	3.5	53	−	+	12.5	A/A2

NEThai, Northeast Thailand; NThai, North Thailand; Phil, Philippines; SThai, Southern Thailand; Mal, Malaysia; CThai, Central Thailand; PCS, parental consanguity; FH, positive family history; NC, nephrocalcinosis; NA, not available.

Table 3 A858D mutations associated with dRTA

SLC4A1 Genotype	Patient no.	References	Ref. case no.	Home	Sex/Age	Family history	$HC0_3$, mmol/l	Cl, mmol/l	K, mmol/l	Creat µmol/l	Rickets	NC	Haemoglobin	
													g/100 ml	Type
A858D/A858D	48	11	1	WIndia	NA/6		NA	NA	4.1	35	+	+	4.9	NA
	49	11	2	WIndia	M/1.3	PCS	NA	NA	NA	27	+	+	4.4	NA
	50	New patients		SEIndia	M/1	Siblings PCS	13	128	3.2	44	+	+	10.4	NA
	51			SEIndia	F/3 identical twins		15	118	4.2	37	+	+	11.0	NA
	52			SEIndia			14	125	3.3	44	+	+	10.5	NA
	53	9	1a	Oman	F/3	Siblings PCS	18	111	4.1	24	–	+	11.1	NA
	54	9	1b	Oman	F/10		15	112	3.0	38	+	–	11.1	NA
	55	9	2	Oman	F/2	PCS	9	113	4	18	+	+	10.9	NA
	56	9	3	Oman	F/2	PCS	13	114	4.1	23	+	–	9.2	NA
	57	9	4a	Oman	M/0.8	Siblings PCS	13	114	4.1	23	+	–	9.4	NA
	58	9	4b	Oman	F/2		11	113	3.8	22	+	–	9.4	NA
	59	9	5	Oman	M/0.8	PCS	9	118	3.2	23	–	+	9.7	NA
A858D/SAO	60	17	RH	Mal	F/15	Siblings	11	121	3.8	NA	NA	+	7.3	A/A2
	61	17	HZ	Mal	F/10		11	114	2.61	NA	NA	+	6.5	A/A2
	62	17	HH	Mal	F/2		10	115	1.9	NA	+	+	7.3	A/A2
	63	7	H:II: 2	Mal	F/0.6		9	NA	2.9	53	+	+	8.5	NA
A858D/ΔV850	64	7	J:II:1	PNG	F/3.6	Siblings	<9	107	2.4	7	+	+	10.7	NA
	65	7	J:II:2	PNG	F/0.4		NA	112	3.6	NA	+	NA	7.7	NA
A858D/G701D	See patients 46 and 47 in Table 2													

WIndia, West India; SEIndia, Southeast India; Mal, Malaysia; PNG, Papua New Guinea; PCS, parental consanguity; FH, positive family history; NC, nephrocalcinosis; NA, not available.

Table 4 ΔV850 mutations associated with dRTA

SLC4A1 genotype	Patient No.	References	Ref case no.	Home	Sex/age	Family history	HCO_3, mmol/l	Cl, mmol/l	K, mmol/l	Creat, μmol/l	Rickets	NC	Haemoglobin g/100 ml
ΔV850/ΔV850	66	7	C:II:1	PNG	M/2		NA	NA	2.3	11	+	–	15.5
	67		New	PNG	F/3.2		NA	NA	1.9	31	+	NA	8.3
	68		New	PNG	M/9.9		NA	120	3.3	33	+	–	14.6
ΔV850/SAO	69	7	D:II:1	PNG	M/1.0		10	121	2.5	7	–	–	11.1
	70	7	E:II:1	PNG	M/19		<9	NA	2.3	11	–	+	11.5
	71	7	F:II:2	PNG	M/1.0		8	NA	1.6	13	+	NA	6.1
	72	7	G:II:1	PNG	M/2		NA	108	3.2	7	+	NA	9.2
	73		New	PNG	F/3.6		11	118	2.8	9	+	NA	12.0
	74		New	PNG	F/5.2		13	115	2.5	17	+	–	10.0
	75		New	PNG	F/8		NA	104	2.9	65	+	NA	12.5
ΔV850/A858D	See patients 64 and 65 in Table 3												

PNG, Papua New Guinea; PCS, parental consanguity; FH, positive family history; NC, nephrocalcinosis; NA, not available.

mutations are similar in many respects to the recessive dRTA caused by H^+-ATPase mutations, rather than to the milder phenotype of dominant dRTA caused by *SLC4A1* mutations. The difference between dRTA caused by recessive and dominant *SLC4A1* mutations is particularly striking in age of presentation, with dRTA from recessive *SLC4A1* mutations usually manifest and diagnosed in early childhood and the dominant disease usually in late childhood or young adults. This may be because in dRTA caused by recessive *SLC4A1* mutations, no functional AE1 is expressed in the kidney, whereas a small amount of functional AE1 may be expressed in dRTA caused by dominant *SLC4A1* mutations. The complications of dRTA (acidosis, hypokalaemia, rickets and nephrocalcinosis) tend to be more severe or have a greater or earlier incidence in recessive dRTA caused by both *SLC4A1* and *ATP6V0A4* or *ATP6V1B1* (coding for H^+-ATPase subunits) mutations than in the dominant disease resulting from *SLC4A1* mutations and may include some loss of kidney function in those with H^+-ATPase mutations.[28]

Red cell haematology

Table 7 presents a summary of haematological data from these dRTA patients. It does not include findings on haematologically symptomless heterozygotes with *SLC4A1* mutations, because in general the blood films of these patients were normal, except in subjects with SAO showing the typical ovalocytic stomatocytes. These morphologic features of SAO were present in all patients with this mutation, regardless of the nature of the mutation on the opposite allele, except for patients 60–63, in whom red cells were smaller, and the predominant morphology was elliptocytes.[7] No red cell fragility studies were made on these patients, but it is possible that the reduced osmotic fragility of SAO red cells, a well-established feature of SAO, was able to compensate for the increased osmotic fragility of the spherocytic red cells in the patients with A858D/SAO. Ovalocytic stomatocytes similar to SAO were found with the homozygous G701D mutation and these cells have sometimes been mistaken for SAO cells. The over-hydrated stomatocytic morphology found in both SAO and homozygous G701D red cells may result from the altered cation permeability of these cells,[29–31] which is discussed later.

Mutations in *SLC4A1* that cause red cell spherocytosis occur at over 40 sites in AE1 (Figure 1), including both cytoplasmic and membrane-spanning sites.[19] Most of the published reports of spherocytic subjects make no mention of urinary acidification, although defective urinary acidification was a feature of a mother and daughter with a large heterozygous C-terminal deletion of AE1 from S477X.[20] Spherocytosis caused by *SLC4A1* mutations often results from degradation of the mutant AE1 and so these mutations are normally found only in heterozygotes. Homozygous mutations have been described, but they can result in complete absence of AE1 and consequently life-threatening haemolytic anaemia and dRTA, as was present in a child with spherocytosis and profound haemolysis caused by V488M.[21] From Table 7 it is clear that the blood films of most of our patients did not contain spherocytes, the main exception being patients with homozygosity of A858D, whose red cells were usually spherocytic, and also acanthocytic, even in freshly drawn blood, as shown in Figure 3. Some spherocytes were present in blood films from patient 1, where the homozygous G701D mutation was complicated by the presence of haemoglobin E, and they were also seen among other morphologically abnormal red cells of patient

Table 5 Other *SLC4A1* mutations associated with dRTA

SLC4A1 Genotype	Patient No.	References	Ref Case No.	Home	Sex/Age	Family history	HCO_3, mmol/l	Cl, mmol/l	K, mmol/l	Creat, µmol/l	Rickets	NC	Haemoglobin	
													g/100 ml	Type
R602H/SAO	76	12	1	SThai	M/5	Siblings	14	112	1.6	88	+	+	15.7	A/A2
	77	12	3	SThai	F/15		20	108	3.8	NA	–	–	NA	NA
Q759H/SAO	78	5	2	Sarawak	M/2		10	107	2.7	62	+	+	4.8	A/A2

SThai, Southern Thailand; PCS, parental consanguity; FH, positive family history; NC, nephrocalcinosis; NA, not available.

Figure 2. Two patients with dRTA and rickets.

Table 6 Comparison of phenotypes in dRTA caused by recessive and dominant *SLC4A1* mutations and H^+-ATPase mutations

Cause of dRTA	Age at diagnosis	Mean plasma HCO_3, mmol/l	Mean plasma K, mmol/l	Patients with rickets (%)	Patients with nephrocalcinosis (%)	References
Recessive tropical *SLC4A1* mutations	4 years (range: 0.5–19 years)	12 (range: 5–21)	2.8 (range: 1.2–4.1)	83	80	This series
Dominant *SLC4A1* mutations	23 years (range: 2–67 years)	19 (range: 5–25)	3.6 (range: 2.1–4.6)	11	58	2, 3
Recessive H^+ATPase (ATP6V1B1, ATP6V0A4) mutations	10 months (range: 0–6 years)	11 (range: 4–18)	3.0 (range: 1.3–4.7)	30	95	24–28

Figures for age and plasma values are not normally distributed, so are given as means and ranges of observations at diagnosis.

Table 7 Hematological findings in dRTA patients

Genotype	Hb, type and other defects	Hb, g/100 ml	Red cell morphology			Haemolysis	
			Normal	Ovalo	Others	Non-acidotic	Acidotic
G701D/G701D, Pts 3, 8, 11, 12, 16, 18–24	A/A2	11.6–14.7	++	+	Stomato, shisto, xero	No	Compensated haemolysis
G701D/G701D, Pts 2, 7, 13, 14	A/E	8.3–12.9	+	++	Stomato, shisto, xero, ellipto, target	No	Compensated haemolysis
G701D/G701D, Pt 1	E/E	11	+	++	Stomato, sphero, xero, target	NA	Haemolytic anaemia
G701D/SAO, Pts 26, 27, 31–33, 43	A/A2	5.9–14.2	+	++	Stomato, shisto, xero, ellipto	No	Compensated haemolysis
G701D/SAO, Pt 34	A/A2, -α 3.7/αα	8.6	Rare	+++	Stomato, shisto, xero	No	Haemolytic anaemia
G701D/SAO, Pt 35	A/A2, G6PD deficient	NA	NA	++	Stomato	No	No
G701D/E522K, Pt 44	NA	4.6	NA	NA	NA	NA	Haemolytic anaemia, regular transfusions
G701D/S773P, Pt 45	NA	13.6	++	++	Stomato	No	No
G701D/A858D, Pts 46,47	A/A2	13.6	+	++	Pincered, shisto, xero	No	Compensated haemolysis
	A/E	12.5	+	++	Pincered, shisto, xero	No	Compensated haemolysis
A858D/A858D, Pts 48–59	NA	4.4–11.0.	+	+	Sphero ++, acantho ++, stomato, shisto, ellipto	Haemolytic anaemia, compensated or on regular tansfusions	Haemolytic anaemia, compensated or on regular tansfusions
A858D/SAO, Pts 60–63	NA	6.5–7.9	NA	+	Ellipto	NA	Haemolytic anaemia
ΔV850/A858D, Pts 64,65	NA	7.7–10.7	+	No	Micro, ellipto, poikilo	NA	Haemolytic anaemia
ΔV850/ΔV850, Pts 66–68	NA	8.3, 14.6, 15.5	normal	No	No	No	No
ΔV850/SAO, Pts 69–75	NA	6.1–12.5	+	+++	Micro, ellipto	NA	Variable
R602H/SAO, Pts 76,77	A/A2	15.7	+	++	Stomato, shisto, aniso, poikilo	No	No
Q759H/SAO, Pt 78	A/A2	4.8–7.4	Rare	+++	Typical SAO	Improved (Hb 11.4)	Haemolytic anaemia, regular transfusions

Abbreviations for acanthocytes, anisocytes, elliptocytes, microcytes, ovalocytes, poikilocytes, shistocytes, spherocytes, stomatocytes and xerocytes. Pt, patient; NA, not available.

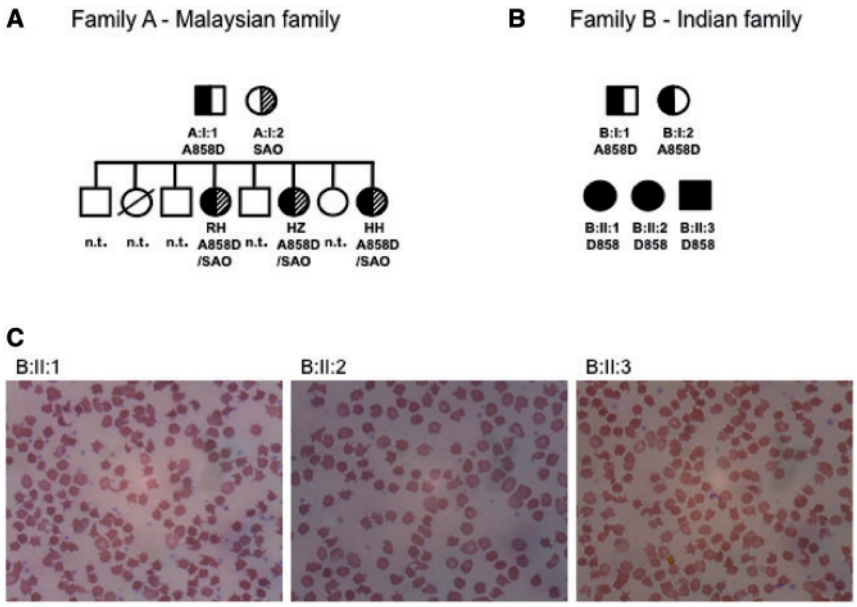

Figure 3. Blood films and pedigrees of patients with the A858D mutation. The pedigrees show the recessive nature of the A858D mutation (solid black denotes D858 and hatch denotes SAO). (**A**) Family tree for the Malaysian family (patients 60–62, Table 3). Both parents are heterozygous for a single mutation and neither parent has dRTA. The three children (RH, HZ and HH) are all compound heterozygotes, D858 is inherited from the father (A:I:1) and SAO is inherited from the mother (A:I:2) and all three have dRTA. (**B**) Family tree for the Indian family (patients 50–52, Table 3). Both parents are heterozygous for a single mutation (A858D) and neither parent has dRTA. The three children (B:II:1, B:II:2 and B:II:3) are all homozygous for D858 and all three have dRTA. (**C**) Blood films from the Indian children (B:II:1, B:II:2 and B:II:3) showing the spherocytic/acanthocytic morphology of the red cells as described previously.[16] n.t., not tested.

44 with E522K/G701D mutations. Spherocytes were also found in a patient with typical dRTA caused by compound heterozygosity of C479W/G701D.[22] In each of these cases, it may be that the mutations A858D, E522K and C479W can be classed as *SLC4A1* spherocytosis mutations, where the mutant AE1 is degraded, effectively giving a null phenotype. Distal RTA will result from any recessive dRTA mutant (e.g. G701D) found as a double heterozygote with any null *SLC4A1* mutant.

Haemolytic anaemia with splenomegaly, hyperbilirubinaemia and reticulocytosis was present in many of these dRTA patients, but in most cases compensated by increased haemopoiesis, although several children with G701D/E522K, Q759H/SAO and homozygous A858D mutations required blood transfusion. A striking observation was that the blood film was normal in patient 66, who was homozygous for ΔV850. Two of the three patients with homozygosity for this mutation had normal haemoglobin levels; the likely explanation for these findings is that this particular *SLC4A1* mutation has only slightly reduced expression in the red cell, as shown by measurements of DIDS-sensitive sulphate flux,[7] unlike the impaired red cell expression of the protein seen in several other dRTA patients with *SLC4A1* mutations.

Several haemoglobinopathies and familial red cell diseases (e.g. thalassaemias, Hb E and glucose 6PD deficiency) are prevalent in parts of the tropics, but with few exceptions (noted in Table 7) their phenotypes were not a feature of the reports included here. Alpha-thalassaemia cannot definitely be excluded without molecular studies, which were not performed on most of our patients, and it might have contributed to the anaemia of some dRTA patients with the ΔV850 genotype, as a high prevalence of this haemoglobin defect has been reported from northern coastal areas of PNG,[23] where these dRTA patients are domiciled.

We have previously observed that systemic acidosis may contribute to the anaemia of dRTA patients with Q759H/SAO, G701D/SAO and homozygous G701D mutations, as shown by a reduced severity of haemolysis during alkali treatment[5,16] and from our current observations comparing the last two columns of Table 7, suggesting that it may play a role in the haemolytic anaemia of dRTA associated with several other *SLC4A1* mutations.

Location of dRTA families

In our earlier work on dRTA, we have referred to patients described in this article as domiciled in

'Southeast Asia'[5,6,16]; however, this usage is not correct, because some of the locations referred to, notably PNG and Oman, are not strictly parts of Southeast Asia. These places all lie between the tropics of Cancer and Capricorn, so we now describe them as 'tropical', contrasting them with the 'temperate' or 'non-tropical' patients and diseases that we have described previously as 'western' or 'occidental'. The word 'tropical' can also refer to several countries in Central and South America, but few patients with any form of dRTA have been reported from these countries.

In Figure 4, we show the geographical domicile of families affected by recessive dRTA caused by three of the most prevalent *SLC4A1* mutations found in the tropics. The most abundant of these is the G701D missense mutation shown in green, most prevalent in Thailand, particularly in the Northeast of this country, but also in several families from the Philippines and Malaysia and in single families in Sarawak and Taiwan. The A858D mutation shown in red has a wider distribution, being found as far west as India and Oman and as far east as PNG. The ΔV850 mutation shown in blue appears to be confined to PNG, where it is the only mutation found so far to account for the high prevalence of recessive dRTA in this country. Figure 4 also shows the area where the prevalence of SAO exceeds 1% of the population.[32]

There are many tropical countries from which cases of dRTA caused by recessive *SLC4A1* mutations have never been reported. These omissions include Myanmar, Vietnam, Cambodia and Laos, but the most striking are Indonesia and China, both with very large populations and adjacent to countries where large numbers of patients with recessive dRTA caused by *SLC4A1* mutations have been reported; however, we have been unable to obtain information on recessive familial dRTA from either country.

Individual *SLC4A1* mutations causing tropical dRTA

Δ400–408 (SAO)

This mutation (Figure 1) was present in 32 of the patients reported here from 27 families (Tables 2–5). In dRTA patients, the SAO mutation was always heterozygous and accompanied by another mutation on the opposite allele, usually G701D, Δ850 or A858D. Homozygous SAO has never been reported and is believed to be lethal *in utero*.[33] SAO in its isolated heterozygous form, without a mutation on the opposite allele, does not cause a urinary acidification defect,[7,14] a finding, together with the absence of homozygous SAO in the population, that has led to the misconception that SAO plays no role in dRTA. In fact, the present series shows that SAO, when present as a compound heterozygote with another *SLC4A1* mutation, is a potent cause of dRTA. This is because SAO AE1 does not transport anions and may not even be expressed in kidney cells, although it is expressed in red cells. Consequently, when SAO is present as a compound heterozygote with a recessive dRTA mutation, it reveals the phenotype of the recessive dRTA mutant.

SAO is widely distributed in SE Asia, as shown in Figure 4, with a particularly high prevalence of up to 35% of the population in the Indonesian island of Sulawesi and the northern coastal region of PNG and a somewhat lower prevalence in the Philippines and other parts of the Malay archipelago and Melanesia. Northern parts of Sumatra and Thailand have a low prevalence. Most of these reports of prevalence were published when the diagnosis of SAO was based on the appearance of the blood film, rather than a genomic analysis, which was not available at the time, and so may be slightly exaggerated owing to the inclusion of other red cell abnormalities with a similar blood film appearance, such as the homozygous G701D mutation.[6,16] Nevertheless, in such areas where SAO is prevalent, such as the Indonesian island of Sulawesi and the northern coastal region of PNG, the possibility of finding recessive dRTA caused by SAO and another mutation on the opposite allele of *SLC4A1* in the population should be higher than in other areas where SAO is rare. SAO is the only *SLC4A1* mutation involved in recessive tropical dRTA that has been found in patients outside the tropics, with isolated reports of SAO cases from countries as far apart as South Africa, Mexico and the UK, although none has so far been reported with dRTA.

The geographical distribution of SAO has suggested that it might have evolved because it protects against malaria, particularly the form caused by *Plasmodium falciparum,* which was formerly prevalent in all areas where a high prevalence of SAO now exists. Several research groups have examined the possibility that SAO has this property, studying both the malarial incidence in populations with a high SAO prevalence and making *in vitro* observations of red cell parasitism by malarial merozoites. The most convincing demonstration that SAO has a malaria-protective effect was the finding of a reduced incidence of cerebral malaria in PNG children with SAO and falciparum infection.[34]

The studies reported here confirm the previous finding that the SAO mutation is invariably

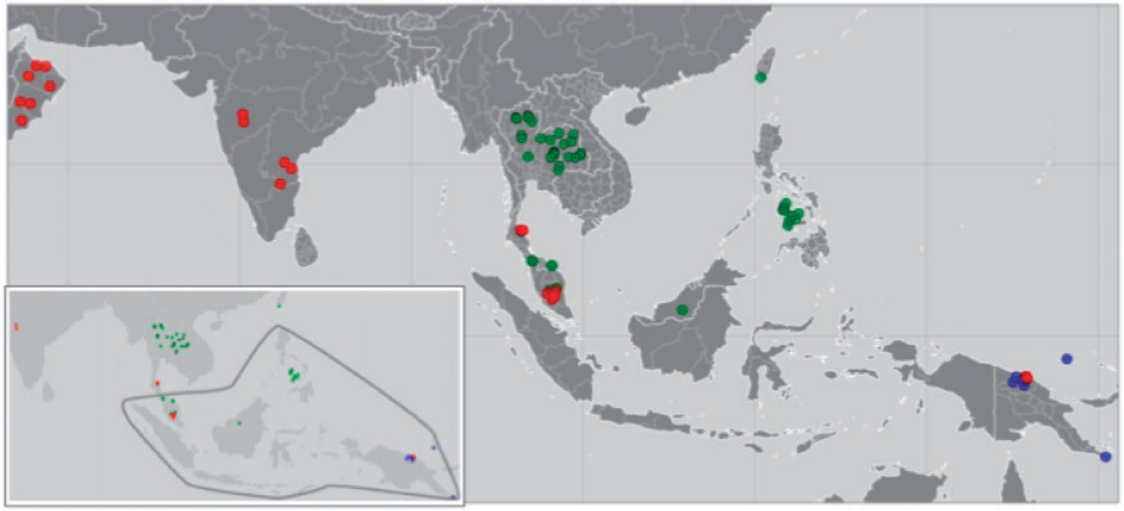

Figure 4. Map of the regions affected in the tropics. Green dots represent G701D cases/families; red dots represent A858D cases/families and blue dots represent ΔV850 cases/families. Inset shows area of SE Asia where SAO is most prevalent.

accompanied by the Memphis mutation, K56E, a benign polymorphism that is common in the general population, especially in the orient,[35] and which does not affect renal H^+ secretion, as it is not present in the truncated renal AE1. Of interest (as discussed later) is the finding that K56E co-exists with the G701D mutation and benign polymorphism, M31T, in patients with dRTA caused by G701D, but it is absent from the G701D-associated dRTA in a western Canadian family,[22] suggesting that the latter may have evolved independently.

G701D

This mutation, a substitution of glycine 701 by aspartic acid (Figure 1), was found in 47 patients from 40 families, the commonest *SLC4A1* mutation found in tropical dRTA. In 21 families, the mutation was homozygous (Table 1); in 19 it was paired as a compound heterozygote with a different *SLC4A1* mutation, usually SAO (Table 2). When appropriate genome studies were made, the G701D mutation was invariably inherited with the non-pathogenic polymorphisms M31T and K56E.[5–7,13] AE1 residues 31 and 56 are within the 65 N-terminal amino acids absent from renal AE1 and so they play no part in the dRTA caused by the G701D mutation.

A population survey found that the G701D genotype had 0.73% prevalence in Northeast (NE) Thailand,[36] and it turned up no instances of this mutation in the population of other parts of the country. In keeping with this observation, the G701D mutation causing dRTA in NE Thailand has usually been homozygous, but in other parts of the tropics, including southern Thailand and Malaysia, dRTA caused by G701D is usually due to compound heterozygosities of G701D with other *SLC4A1* mutations. In the Philippines, the situation is intermediate: two of the seven dRTA families reported were homozygous for G701D, the remaining five had compound heterozygotes of G701D/SAO.[6] The G701D mutation has not so far been found in PNG (Figure 4).

The complex triad of mutations (G701D/M31T/K56E) found in tropical dRTA suggests a founder effect and so far has not been reported outside the tropics. However, a G701D mutation has been demonstrated in a patient of Scandinavian ancestry, now resident in Alberta, who has familial spherocytosis associated with the unique mutation, C479W, and typical dRTA caused by compound heterozygosity of C479W/G701D.[22] In this patient, the G701D mutation is not accompanied by the K56E polymorphism (the report does not mention M31T), suggesting the mutation that gave rise to G701D in this family arose independently of the triad of mutations responsible for the complex genotype G701D/M31T/K56E in tropical families. Red cells in G701D heterozygotes have normal morphology[16]; however, G701D homozygosity gives rise to oval red cells that are similar to those of SAO, except that the slits ('stomata') and transverse haemoglobin ridges, and occasional macrocytes typical of SAO are seen less commonly.[6,15,16]

A858D

This mutation, a substitution of alanine 858 by aspartic acid (Figure 1), caused dRTA in 20 patients

from 12 families (Table 3). The mutation is geographically widespread (Figure 4), with the most recent reports emanating from Oman (seven patients in five families) and India (five patients in three families), where all children with the mutation have been homozygotes and all except one are children of consanguineous marriages. Earlier reports of dRTA patients with this mutation were from PNG, Malaysia and South Thailand; all these cases were heterozygotes of the mutation with some other *SLC4A1* mutation, usually SAO.

The first report of dRTA caused by this mutation described it as 'dominant'[7] and in this respect differing from other forms of tropical dRTA described here. The evidence for dominant transmission came from a study of two families with dRTA caused by the compound heterozygotes of A858D with SAO (patient 63) and A858D with ΔV850 (patients 64 and 65). Neither parent carrier with the A858D mutation was acidotic, so they were subjected to the furosemide/fludrocortisone test of urinary acidification.[37] In both cases, the A858D carrier failed to produce urine more acid than pH 6.5 and so fulfilled the accepted criteria for dominant dRTA in its 'incomplete form'.[38] Subsequent reports of dRTA patients with the A858D mutation contain no details of the parents' urinary acidification ability and did not distinguish between dominant and recessive disease,[10] although the report of two unrelated Indian dRTA patients who were homozygous for the mutation[11] was more in keeping with recessive than dominant transmission.

We have been able to perform a gene analysis in a Malaysian family (family tree in Figure 3), with three dRTA siblings, patients 60–62 in Table 3, previously described as having 'dRTA and hereditary elliptocytosis'.[17] These patients have proved to be compound heterozygotes of A858D/SAO, inheriting A858D from their father and SAO from their mother. Neither parent has dRTA; the father with the heterozygous A858D acidifies his urine normally to pH 5.0 on testing with furosemide/fludrocortisone. The A858D mutation in this family shows typical recessive transmission, disagreeing with the earlier classification of the A858D mutation as 'dominant'.[7] One explanation for this discrepancy is that the mutation differs in its clinical penetration in different families, depending on factors that are not understood. Another possibility is that the compliance of the parents in the earlier studies was incomplete, as the self-administration of furosemide and fludrocortisone by these subjects was unsupervised.

The recent discovery of multiple Indian and Omani families with dRTA caused by the A858D mutation[9,11] (Table 3) suggests that there may be many patients with this mutation in these countries, where familial dRTA has often been reported without details of the *SLC4A1* genotype.

ΔV850

This mutation, a deletion of valine 850 (Figure 1), was found in 12 patients representing 11 families (Table 4). The mutation was homozygous in three patients from different families and present as a compound heterozygote in the other eight families, in one family with A858D and in the other seven families with the SAO mutation. All patients with the ΔV850 mutation were domiciled in PNG, 8 of the 11 in the north coastal province of East Sepik (Figure 4). The mutation has not so far been found in any patient from outside PNG, so it has probably evolved in this island, where the Melanesian population is ethnically quite different from the people of Sino/Malay or Arab ethnicity in other tropical countries mentioned so far.

The phenotype of dRTA patients with this mutation does not differ from that of dRTA patients in other parts of the tropics and usually presents with failure to thrive and rickets (Figure 2), except for slightly more severe hypokalaemia. Episodes of hypokaleamic paresis, unremarked in our other tropical cases of dRTA, occurred in five patients (patients 64, 66, 68, 70 and 73) and contributed to a fatal outcome in patients 64, 66 and 70. Initial levels of plasma potassium concentration (mean 2.61 ± 0.62 mmol/l) were slightly lower than in other tropical dRTA patients (2.93 ± 0.74 mmol/l), but the difference was not significant. Marked hypokalaemia with hypokaleamic paresis has been described in several other patients with tropical dRTA from both PNG[39] and Thailand,[40] but these early reports have not provided genetic information on the cause of dRTA.

Some heterozygous healthy siblings of patients with the ΔV850 mutation also carried the D38A (Darmstadt) mutation on the opposite allele,[7] but for simplicity the details are not included in Table 4, as this mutation is known to be harmless and residue 38 is not present in the truncated renal form of AE1.

Patients with the ΔV850 mutation also differ from other tropical dRTA patients in that the homozygous state did not cause haemolytic anaemia: two of the three patients with this genotype having normal haemoglobin levels and red cells with normal morphology; this correlated with the relatively normal expression of AE1 in red cells, as shown by their disodium 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS)-sensitive sulphate flux.[7]

Other mutations associated with dRTA

The missense mutations E522K (substitution of glutamic acid 522 by lysine), R602H (substitution of arginine 602 by histidine), Q759H (substitution of glutamine 759 by histidine) and S773P (substitution of serine 773 by proline) were all found in single families: R602H in two siblings, the other mutations in single children (Table 5). All dRTA patients were compound heterozygotes of the mutation in question: R602H and Q759H with SAO, E522K and S773P with G701D. Three of the four families involved were domiciled in sites that so far have produced few or no other dRTA patients with *SLC4A1* mutations: E522K in Taiwan, R602H in South Thailand and Q759H in an Iban child in a remote part of Sarawak, suggesting that their unique mutation might be representative of a local population nidus that had so far escaped clinical detection. The dRTA phenotype of these patients was similar to that of other dRTA patients reported here, except that patient 78 with Q759H/SAO had a particularly severe haemolytic anaemia that responded to alkali treatment, although marked growth retardation persisted even after acidosis was corrected by alkali therapy.[5]

Expression studies of *SLC4A1* mutations

Expression studies have revealed a variety of defects in the different *SLC4A1* mutations described in this article. Studies with the *Xenopus* oocyte expression system have shown that the SAO mutation cannot mediate chloride transport and that the A858D and ΔV850 mutations both have markedly impaired chloride transport.[7] With the G701D mutation, chloride transport is also absent except in the presence of glycophorin A (GPA),[7,13] a single-spanning red cell membrane protein not found in the renal alpha-intercalated cell; the presence of GPA also enhances chloride transport in oocytes expressing the ΔV850 and A858D mutations.

When expressed in polarized Madin-Darby canine kidney (MDCK) cells, normal AE1 is for the most part correctly targeted to the basolateral membrane. However, when present as homozygous or compound heterozygous *SLC4A1* mutations, the dimer or hetero-oligomer formed between the AE1 mutants is misfolded or dysfunctional and unable to traffic to the cell membrane and it is retained and degraded in the cytosol. In contrast, when accompanied as heterozygotes with normal AE1, the recessive mutations SAO, G701D, S773D, ΔV850 and A858D are 'rescued' in dimer or tetramer form by the normal AE1 protein and correctly trafficked to the basolateral membrane. Thus, normal AE1 protein exhibits a 'dominant positive' effect over the mutant AE1 proteins.[41] This pattern of trafficking by recessive dRTA mutations differs from that of dominant dRTA mutations (not considered here), which in general show essentially normal anion transport in *Xenopus* oocytes, but fail to traffic normally in polarized renal tubular cells. Dominant dRTA mutations forming hetero-oligomers with normal AE1 are either retained intracellularly or mis-trafficked to the apical membrane, a 'dominant negative' effect. The molecular mechanisms of dominant and recessive dRTA phenotypes attributable to *SLC4A1* mutations have been elucidated and are explained by the trafficking properties of the homodimers and heterodimers of the wild-type and mutant AE1 proteins to the basolateral membrane and their ability to mediate chloride/bicarbonate exchange.[41]

Cation leak in tropical dRTA

Interestingly, several *SLC4A1* mutations also cause AE1 to act as a monovalent cation conductance, causing a 'cation leak' of sodium and potassium. This was first observed in several hereditary stomatocytosis mutations (Figure 1) that caused a cold-induced red cell cation leak and a similar leak in *Xenopus* oocytes expressing the corresponding AE1 mutants, estimated by rubidium, $^{86}Rb^+$, influx and direct intracellular cation measurements.[42] Similar cation transport experiments were undertaken in *Xenopus* oocytes expressing the non-tropical autosomal dominant dRTA-causing AE1 mutants R589H, G609R and S613F and the tropical autosomal recessive mutant G701D. All these mutants demonstrated a cation leak ($^{86}Rb^+$ influx) at 0°C, which was much greater in the tropical G701D mutant.[31] At 22°C, the autosomal dominant mutants had an $^{86}Rb^+$ leak no greater than water-injected oocytes, but the G701D mutant continued to show a large rubidium leak. This leak was also demonstrated electrophysiologically and by direct intracellular cation measurements. This difference was investigated further by examining the cation leak property of other tropical autosomal recessive dRTA-causing AE1 mutants (S773P, Δ850 and A858D) by lithium (Li^+) influx: G701D had a large Li^+ influx, similar to the other tropical dRTA mutants, and much greater than the baseline cation leak seen in water-injected oocytes.[30] The authors concluded that cation leakiness was a consistent property of tropical dRTA-causing AE1 mutations. Intriguingly, the same holds true for

SAO in which a large cation leak was demonstrated recently in SAO erythrocytes and in *Xenopus* oocytes expressing SAO AE1.[43]

Discussion

Our report and review emphasizes the haematological and genetic differences between the dRTA caused by *SLC4A1* mutations in temperate countries and the disease found in the tropics. In temperate countries, the disease is relatively uncommon: fewer than 20 families have been reported. In most cases, the disease is dominant[4] and usually caused by a substitution of R589 and less often by a deletion or addition to the C- terminal of AE1. Red cells usually have normal morphology and haemolytic anaemia is not a feature. Recessive dRTA caused by *SLC4A1* mutations is rare in temperate countries: only three unrelated patients have been described and each of these involves an *SLC4A1* spherocytosis mutation that gave very low expression of AE1 or a null phenotype: a child with homozygous V488M in Portugal,[21] a child with homozygous S667F in Algeria[44] and a Scandinavian patient with G479W/G701D who is domiciled in Canada.[22]

In the tropics, dRTA caused by *SLC4A1* mutations is quite different. Dominant dRTA is virtually absent; the exception to this generalization has been the discovery in a Thai child with dRTA of a *de novo* R589 substitution,[45] one of the commonest dRTA-causing mutations seen in the West. All other tropical cases of dRTA caused by *SLC4A1* mutations are recessive and summarized in our report of 62 families comprising 78 dRTA patients. None of the eight different *SLC4A1* mutations reported here from the tropics has been found in dRTA patients from outside the tropics. These geographical differences are marked and require explanation.

The high prevalence of recessive dRTA in the tropics suggests the existence of an environmental factor that has favoured the local evolution of these *SLC4A1* mutations. A possible explanation for selective evolution is that the mutations concerned provide some protection against malaria. The main points in favour of this hypothesis are (i) the areas of the tropics where patients with these mutations are found all have a high prevalence of malaria or have had so in recent times, particularly of the most virulent *Plasmodium falciparum* variety; (ii) several other recessive familial diseases have evolved, or are thought to have evolved, in malarious areas of the world, because of the protection they provide against malaria, the best known being sickle cell (HbS) disease—others include the thalassaemias, G6PD deficiency and various haemoglobinopathies, particularly HbC and HbE (for review see[46,47]); (iii) all the familial diseases known, or postulated, to protect against malaria affect the red cell, the major host cell in the body to suffer invasion by the malaria parasite and a cell affected chemically or structurally by all the *SLC4A1* mutations described here and (iv) SAO, caused by the *SLC4A1* mutation Δ400–408 is a major contributor to the compound heterozygotes causing tropical dRTA and several lines of evidence suggest that it is protective against malaria infection,[48–50] the most convincing being the demonstration in a malarious area of PNG that its presence provides protection against cerebral malaria.[34]

If *SLC4A1* mutations that cause tropical dRTA have a protective effect against malaria, one might expect a similar protective effect from some of the many *SLC4A1* mutations that cause morphological red cell changes, but which are not associated with dRTA, including the several established mutations that cause hereditary spherocytosis.[19] However, none of these mutations has shown a geographic tropical preponderance,[19] suggesting that a malaria-protective effect of *SLC4A1* mutations is present only in those mutations associated with recessive dRTA. An explanation for this apparent specificity is that it might result from the monovalent cation leakiness that has recently been shown to exist in cells carrying AE1 with these dRTA mutations.

As already discussed, cation leakiness is a feature of the tropical recessive dRTA-causing mutants[30,31] and SAO[43] and only a minor feature of occidental dominant dRTA mutants. The monovalent cation leakiness of tropical recessive dRTA mutants has been shown mainly in *Xenopus* oocyte expression studies, but it is not clear how relevant the oocyte findings are to cation movements in the red cell, although the SAO mutation that is associated with dRTA in tropical Asia has been shown to have this effect in both the oocyte and red cell[29]; moreover, G701D/SAO cells show an even larger cold-induced cation leak in red cells.[43] However, changes in sodium and potassium content of the red cell that normally harbours the reproductive stage of the malaria parasite's life cycle seem likely to be damaging to development of the parasite[51,52] and could contribute to a malaria-protective effect by prematurely shortening the parasite's intracellular incubation. Interestingly, *Plasmodium falciparum* parasite invasion and growth are reduced in dehydrated or dense red cells.[53] Clearly, there is a need for more work in this area, particularly on the red cells affected by heterozygous mutations, as the cation leakiness that might be malaria protective should be evident in these cells and not just in the

red cells of subjects with homozygous or compound heterozygous dRTA mutations, where the adverse clinical effects of dRTA may offset any survival advantage caused by *SLC4A1* mutations.

Funding

S Khositseth would like to acknowledge funding support from a Research Scholar Grant (RMU5380032) from the Thailand Research Fund.

Conflict of interest: None declared.

References

1. Tanner MJ. The structure and function of band 3 (AE1): recent developments (review). *Mol Memb Biol* 1997; **14**:155–65.
2. Bruce LJ, Cope DL, Jones GK, Schofield AE, Burley M, Povey S, *et al.* Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene. *J Clin Invest* 1997; **100**:1693–707.
3. Wrong O, Unwin R, Cohen E, Tanner M, Thakker R. Unravelling of the molecular mechanisms of kidney stones. Report of a Meeting of Physicians and Scientists. *Lancet* 1996; **348**:1561–5.
4. Sritippayawan S, Kirdpon S, Vasuvattakul S, Wasanawatana S, Susaengrat W, Waiyawuth W, Nimmannit S, Malasit P, Yenchitsomanus P-T. A de novo R589C mutation of anion exchanger 1 causing distal renal tubular acidosis. *Pediat Nephrol* 2003; **18**:644–8.
5. Choo KE, Nicoli TK, Bruce LJ, Tanner MJ, Ruiz-Linares A, Wrong OM. Recessive distal renal tubular acidosis in Sarawak caused by AE1 mutations. *Pediatr Nephrol* 2006; **21**:212–7.
6. Anacleto FE, Bruce LJ, Clayton P, Hegde S, Resontoc LP, Wrong O. Distal renal tubular acidosis in Filipino children, caused by mutations of the anion-exchanger SLC4A1 (AE1, Band 3) gene. *Nephron Physiol* 2010; **114**:19–24.
7. Bruce LJ, Wrong O, Toye AM, Young MT, Ogle G, Ismail Z, *et al.* Band 3 mutations, renal tubular acidosis and South-East Asian ovalocytosis in Malaysia and Papua New Guinea: loss of up to 95% band 3 transport in red cells. *Biochem J* 2000; **350**:41–51.
8. Chang YH, Shaw CF, Jian SH, Hsieh KH, Chiou YH, Lu PJ. Compound mutations in human anion exchanger 1 are associated with complete distal renal tubular acidosis and hereditary spherocytosis. *Kidney Int* 2009; **76**:774–83.
9. Fawaz NA, Beshlaw IO, Al Zadjali SA, Al Ghaithi HK, Elnaggari MA, Elnour I, *et al.* dRTA and haemolytic anaemia: first description of SLC4A1 A858D mutation in homozygous state. *Eur J Haematol* 2011; **88**:350–5.
10. Khositseth S, Sirikanerat A, Wongbenjarat K, Opastirakul S, Khoprasert S, Peuksungnern R, *et al.* Distal renal tubular acidosis associated with anion exchanger 1 mutations in children in Thailand. *Am J Kidney Dis* 2007; **49**:841–50.
11. Shmukler BE, Kedar PS, Warang P, Desai M, Madkaikar M, Ghosh K, *et al.* Hemolytic anemia and distal renal tubular acidosis in two Indian patients homozygous for SLC4A1/AE1 mutation A858D. *Am J Hematol* 2010; **85**:824–8.
12. Sritippayawan S, Sumboonnanonda A, Vasuvattakul S, Keskanokwong T, Sawasdee N, Paemanee A, *et al.* Novel compound heterozygous SLC4A1 mutations in Thai patients with autosomal recessive distal renal tubular acidosis. *Am J Kidney Dis* 2004; **44**:64–70.
13. Tanphaichitr VS, Sumboonnanonda A, Ideguchi H, Shayakul C, Brugnara C, Takao M, *et al.* Novel AE1 mutations in recessive distal renal tubular acidosis. Loss-of-function is rescued by glycophorin A. *J Clin Invest* 1998; **102**:2173–9.
14. Vasuvattakul S, Yenchitsomanus PT, Vachuanichsanong P, Thuwajit P, Kaitwatcharachai C, Laosombat V, *et al.* Autosomal recessive distal renal tubular acidosis associated with Southeast Asian ovalocytosis. *Kidney Int* 1999; **56**:1674–82.
15. Yenchitsomanus PT, Vasuvattakul S, Kirdpon S, Wasanawatana S, Susaengrat W, Sreethiphayawan S, *et al.* Autosomal recessive distal renal tubular acidosis caused by G701D mutation of anion exchanger 1 gene. *Am J Kidney Dis* 2002; **40**:21–9.
16. Khositseth S, Sirikanaerat A, Khoprasert S, Opastirakul S, Kingwatanakul P, Thongnoppakhun W, *et al.* Hematological abnormalities in patients with distal renal tubular acidosis and hemoglobinopathies. *Am J Hematol* 2008; **83**:465–71.
17. Thong MK, Tan AA, Lin HP. Distal renal tubular acidosis and hereditary elliptocytosis in a single family. *Singapore Med J* 1997; **38**:388–90.
18. Bruce LJ. Hereditary stomatocytosis and cation-leaky red cells—recent developments. *Blood Cell Mol Dis* 2009; **42**:216–22.
19. Stewart AK, Kurschat CE, Alper CL. *The SLC4 Anion Exchange Gene Family. Seldin and Giebisch's The Kidney*. 3rd edn. New York, Elsevier, 2008:1499–537.
20. Rysava R, Tesar V, Jirsa M Jr, Brabec V, Jarolim P. Incomplete distal renal tubular acidosis coinherited with a mutation in the band 3 (AE1) gene. *Nephrol Dial Transplant* 1997; **12**:1869–73.
21. Ribeiro ML, Alloisio N, Almeida H, Gomes C, Texier P, Lemos C, *et al.* Severe hereditary spherocytosis and distal renal tubular acidosis associated with the total absence of band 3. *Blood* 2000; **96**:1602–4.
22. Chu C, Woods N, Sawasdee N, Guizouarn H, Pellissier B, Borgese F, *et al.* Band 3 Edmonton I, a novel mutant of the anion exchanger 1 causing spherocytosis and distal renal tubular acidosis. *Biochem J* 2010; **426**:379–88.
23. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bull World Health Org* 2001; **79**:704–12.
24. Karet FE, Finberg KE, Nayir A, Bakkaloglu A, Ozen S, Hulton SA, *et al.* Localization of a gene for autosomal recessive distal renal tubular acidosis with normal hearing (rdRTA2) to 7q33–34. *Am J Hum Genet* 1999; **65**:1656–65.
25. Karet FE, Finberg KE, Nelson RD, Nayir A, Mocan H, Sanjad SA, *et al.* Mutations in the gene encoding B1 subunit of H^+-ATPase cause renal tubular acidosis with sensorineural deafness. *Nat Genet* 1999; **21**:84–90.
26. Smith AN, Skaug J, Choate KA, Nayir A, Bakkaloglu A, Ozen S, *et al.* Mutations in ATP6N1B, encoding a new kidney vacuolar proton pump 116-kD subunit, cause

recessive distal renal tubular acidosis with preserved hearing. *Nat Genet* 2000; **26**:71–5.

27. Vargas-Poussou R, Houillier P, Le Pottier N, Strompf L, Loirat C, Baudouin V, *et al.* Genetic investigation of autosomal recessive distal renal tubular acidosis: evidence for early sensorineural hearing loss associated with mutations in the ATP6V0A4 gene. *J* Am Soc Nephrol 2006; **17**:1437–43.
28. Vivante A, Lotan D, Pode-Shakked N, Landau D, Svec P, Nampoothiri S, *et al.* Familial autosomal recessive renal tubular acidosis: importance of early diagnosis. *Nephron Physiol* 2011; **119**:31–9.
29. Bruce LJ, Ring SM, Ridgwell K, Reardon DM, Seymour CA, Van Dort HM, *et al.* South-east asian ovalocytic (SAO) erythrocytes have a cold sensitive cation leak: implications for in vitro studies on stored SAO red cells. *Biochim Biophys Acta* 1999; **1416**:258–70.
30. Walsh S, Borgese F, Gabillat N, Guizouarn H. Southeast Asian AE1 associated renal tubular acidosis: cation leak is a class effect. *Biochem Biophys Res Commun* 2009; **382**:668–72.
31. Walsh SB, Borgese F, Gabillat N, Unwin RJ, Guizouarn H. Cation transport activity of anion exchanger 1 (AE1) mutations found in inherited distal renal tubular acidosis (dRTA): structure-function implications for AE1. *Am J Physiol* 2008; **295**:F343–50.
32. Fleming AF. Haematological diseases of the tropics. In: Cook GC, ed. *Manson's tropical diseases*. Philadelphia, Elsevier Science, 2009.
33. Liu SC, Jarolim P, Rubin HL, Palek J, Amato D, Hassan K, *et al.* The homozygous state for the band 3 protein mutation in Southeast Asian Ovalocytosis may be lethal. *Blood* 1994; **84**:3590–1.
34. Allen SJ, O'Donnell A, Alexander ND, Mgone CS, Peto TE, Clegg JB, *et al.* Prevention of cerebral malaria in children in Papua New Guinea by southeast Asian ovalocytosis band 3. *Am J Trop Med Hygiene* 1999; **60**:1056–60.
35. Jarolim P, Palek J, Amato D, Hassan K, Sapak P, Nurse GT, *et al.* Deletion in erythrocyte band 3 gene in malaria-resistant Southeast Asian ovalocytosis. *Proc Natl Acad Sci USA* 1991; **88**:11022–6.
36. Yenchitsomanus PT, Sawasdee N, Paemanee A, Keskanokwong T, Vasuvattakul S, Bejrachandra S, *et al.* Anion exchanger 1 mutations associated with distal renal tubular acidosis in the Thai population. *J Hum Genet* 2003; **48**:451–6.
37. Walsh SB, Shirley DG, Wrong OM, Unwin RJ. Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. *Kidney Int* 2007; **71**:1310–6.
38. Wrong O, Davies HE. The excretion of acid in renal disease. *Q J Med* 1959; **28**:259–313.
39. Erasmus RT, Lavu EK, Savory J, Wills M. Hypokalaemic paralysis associated with renal tubular acidosis. *P N G Med J* 1997; **40**:173–6.
40. Nilwarangkur S, Nimmannit S, Chaovakul V, Susaengrat W, Ong-aj-Yooth S, Vasuvattakul S, *et al.* Endemic primary distal renal tubular acidosis in Thailand. *Q J Med* 1990; **74**:289–301.
41. Yenchitsomanus PT, Kittanakom S, Rungroj N, Cordat E, Reithmeier RA. Molecular mechanisms of autosomal dominant and recessive distal renal tubular acidosis caused by SLC4A1 (AE1) mutations. *J Mol Genet Med* 2005; **1**:49–62.
42. Bruce LJ, Robinson HC, Guizouarn H, Borgese F, Harrison P, King MJ, *et al.* Monovalent cation leaks in human red cells caused by single amino-acid substitutions in the transport domain of the band 3 chloride-bicarbonate exchanger, AE1. *Nat Genet* 2005; **37**:1258–63.
43. Guizouarn H, Borgese F, Gabillat N, Harrison P, Goede JS, McMahon C, *et al.* South-east Asian ovalocytosis and the cryohydrocytosis form of hereditary stomatocytosis show virtually indistinguishable cation permeability defects. *B J Haematol* 2011; **152**:655–64.
44. Toye AM, Williamson RC, Khanfar M, Bader-Meunier B, Cynober T, Thibault M, *et al.* Band 3 Courcouronnes (Ser667Phe): a trafficking mutant differentially rescued by wild-type band 3 and glycophorin A. *Blood* 2008; **111**:5380–9.
45. Sritippayawan S, Kirdpon S, Vasuvattakul S, Wasanawatana S, Susaengrat W, Waiyawuth W, *et al.* A de novo R589C mutation of anion exchanger 1 causing distal renal tubular acidosis. *Pediatr Nephrol* 2003; **18**:644–8.
46. Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. *Am J Hum Genet* 2005; **77**:171–92.
47. Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, *et al.* Malaria and the red cell. *Hematol Am Soc Hematol Educ Prog* 2002; 35–57.
48. Cattani JA, Gibson FD, Alpers MP, Crane GG. Hereditary ovalocytosis and reduced susceptibility to malaria in Papua New Guinea. *Trans Royal Soc Tropic Med Hygiene* 1987; **81**:705–9.
49. Foo LC, Rekhraj V, Chiang GL, Mak JW. Ovalocytosis protects against severe malaria parasitemia in the Malayan aborigines. *Am J Trop Med Hygiene* 1992; **47**:271–5.
50. Genton B, al-Yaman F, Mgone CS, Alexander N, Paniu MM, Alpers MP, *et al.* Ovalocytosis and cerebral malaria. *Nature* 1995; **378**:564–5.
51. Lew VL, Tiffert T, Ginsburg H. Excess hemoglobin digestion and the osmotic stability of Plasmodium falciparum-infected red blood cells. *Blood* 2003; **101**:4189–94.
52. Staines HM, Ellory JC, Kirk K. Perturbation of the pump-leak balance for Na(+) and K(+) in malaria-infected erythrocytes. *Am J Physiol* 2001; **280**:C1576–87.
53. Tiffert T, Lew VL, Ginsburg H, Krugliak M, Croisille L, Mohandas N. The hydration state of human red blood cells and their susceptibility to invasion by Plasmodium falciparum. *Blood* 2005; **105**:4853–60.