

## Distal Renal Tubular Acidosis with Hereditary Spherocytosis

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Hereditary spherocytosis (HS) and distal renal tubular acidosis (dRTA), although distinct entities, share the same protein *i.e.* the anion exchanger1 (AE1) protein. Despite this, their coexistence has been rarely reported. We hereby describe the largest family to date with coexistence of dRTA and HS and discuss the molecular basis for the co-inheritance of these conditions.

**Keywords:** Distal renal tubular acidosis, Familial, Hemolysis, Spherocytosis,

**B**and 3, also known as anion exchange 1 (AE1), is a membrane glycoprotein that mediates chloride/ bicarbonate exchange. It is encoded by the human solute carrier family 4 anion exchange member 1 (*SLC4A1*) gene, which is expressed both at the red blood cell membrane (eAE1) and at the basolateral membrane of alpha intercalated cells in the distal tubules of the kidney (kAE1) [1, 2]. Mutations in *SLC4A1* may cause both a renal acidification defect manifesting as distal renal tubular acidosis (dRTA), as well as red cell dysmorphism which include hereditary spherocytosis, hereditary stomatocytosis and South East Asian ovalocytosis [3]. The co-existence of dRTA and ovalocytosis is common in certain geographical areas; however, co-existence of dRTA with hereditary spherocytosis has rarely been reported [4-8]. Here we report 3 siblings in a Southern Indian family with dRTA and hereditary spherocytosis. The patients described here were listed in a review of tropical dRTA [9], but a full description of these unusual cases has not been reported.

### CASE REPORT

The first of the twin, offspring of non-consanguineous parentage, presented at 2 years and 10 months of age with failure to thrive and features of rickets. Evaluation, including frusemide challenge test, showed normal vitamin D levels and renal functions, normal anion gap hypokalemic metabolic acidosis, with high urine pH (pH 6.0 following frusemide administration), hypercalciuria and nephrocalcinosis and was conclusive of dRTA. Complete blood count revealed compensated hemolysis (reticulocyte count 5.4%, hemoglobin level 10.5 g/dL) with presence of spherocytes and acanthocytes. The diagnosis of hereditary spherocytosis was confirmed by

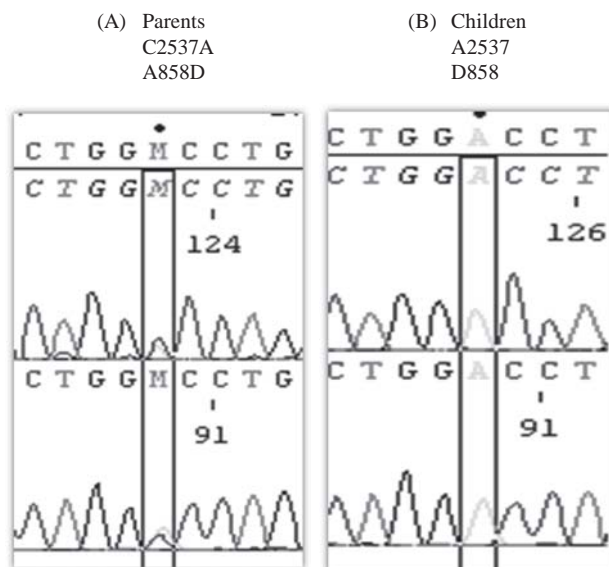
positive osmotic fragility test. Her twin sister was consulted shortly afterwards, with similar complaints and clinical picture and was also found to have dRTA (normal anion gap metabolic acidosis with urine pH of 6.1 following frusemide challenge) and hereditary spherocytosis (reticulocyte count 4.7%, hemoglobin 9.1 g/dL and spherocytes and acanthocytes).

Both children responded to alkali supplementation (2.5 to 3 mmol/Kg of bicarbonate) with normalisation of blood chemistry and significant improvement of their growth. Their younger sibling, 14-months-old boy, evaluated for poor weight gain, was found to have similar clinical features and was also diagnosed to have dRTA (hypokalemic metabolic acidosis, with high urine pH); and hereditary spherocytosis. Physical growth and development improved significantly, following alkali therapy, within weeks.

The gene encoding AE1 (*SLC4A1*) was analysed (**Fig. 1**) and a c2573a mutation found, resulting in the substitution of alanine by aspartic acid, Ala858Asp. All patients were homozygous for this substitution while their parents were heterozygous for the same mutation. The parents had clinically normal blood picture and renal functions.

### DISCUSSION

AE1 is the most abundant protein on the red cell membrane and consists of an N-terminal cytoplasmic domain (1-399), that interacts with ankyrin in the red cell cytoskeleton and maintains its biconcave disc shape, and a C-terminal, membrane-spanning domain (400-911) which is responsible for chloride-bicarbonate exchange [1]. A truncated form of AE1, lacking the first 65 N-



**Fig. 1** DNA sequencing of *SLC4A1* shows that the parents have the heterozygous mis-sense mutation C2537A which leads to amino acid substitution A858D (A) whereas their children were homozygous for the same point mutation A2537/D858 (B).

terminal amino acids is present on the basolateral membrane of the alpha-intercalated cells of the renal collecting duct where it plays an important role in acid secretion [2,3]. Consequently mutations in *SLC4A1* which codes for the AE1 protein may have a pleiotropic effect causing both dRTA as well as red cell dysmorphology. However the occurrence of both effects from a single mutation is rare and was first reported in a severe transfusion dependent patient with spherocytosis where homozygous mutations in *SLC4A1* caused complete or very severe loss of AE1 (AE1<sub>null</sub>) [5]. The current report discusses the largest family to date of combined dRTA and spherocytosis secondary to a homozygous *SLC4A1* mutation. This is the second such report from India [7] but hails from a geographically distinct area (South India) than those previously reported (West India).

The rarity of the coexistence of both HS and dRTA in the same patient despite sharing a common protein has been a source of various postulations. Presence of glycophorin A in the red cells in contrast to its absence in the alpha – intercalated cells has been postulated as one of the explanations. Glycophorin acts as a chaperone and improves eAE1 trafficking to the red cell membrane. Mutations in *SLC4A1* that specifically cause dRTA affect trafficking of kAE1 in the internal membranes, kAE1 is either held up in the endoplasmic reticulum or Golgi body or delivered to the wrong plasma membrane, i.e. the

apical membrane instead of the basolateral membrane in alpha-intercalated cells [10].

Apart from the AE1<sub>null</sub> described above, homozygous mutations, resulting in both spherocytosis and dRTA, had been rarely reported till the description of the homozygous mutation Ala858Asp in 2010 [7]. This was first reported in two children from different families but from the same Maratha ethnic group in Mumbai from Western India. Subsequently it has also been reported from Oman [8]. Similar to these reports all the present patients presented at an early age with features of dRTA and showed significant reticulocytosis. The most characteristic features of the blood film were the preponderance of spherocytes and acanthocytes, as reported previously [7,8].

The Ala858Asp mutation is effectively a mild mutation. A previous report showed that heterozygous Ala858Asp individuals express 80% AE1 in their red cell membranes, compared to normal controls, not low enough to cause hereditary spherocytosis [4]. However homozygous Ala858Asp patients will therefore have d' 60% AE1, a point at which the membrane becomes unstable and hereditary spherocytosis ensues.

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## Locked-in Syndrome as a Presentation of Snakebite

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Snake bite is a common condition in tropical countries. Neurotoxic features of snake bite vary from early morning neuroparalytic syndrome to various cranial nerve palsies. Locked in syndrome (LIS) is a rare presentation. We present four children that had LIS; three patients had total and one had incomplete LIS. All patients made successful recovery with polyvalent anti-snake venom and supportive management. This case series highlights the importance of early diagnosis of LIS in snake bite.

**Key words:** Children, Locked in syndrome, Snakebite.

Snake envenomation is a major cause of mortality and morbidity all over the world but more so in developing countries because of lack of advanced life support equipment [1]. Various presentations of snake bite may include neuromuscular paralysis, bleeding disorder, renal failure and so on. Neurological manifestations are caused by elapidæ group (Cobras, kraits). The common krait is nocturnally active snake with painless bite; so many patients with neurological manifestations present to emergency without history of snake bite [2]. In 60%-70% of cases, snakebite occurs when the patients were asleep and site of bite is undetectable in 17% cases [3]. Thus, high degree of suspicion is required in such cases to reach at a diagnosis.

Locked in syndrome (LIS) is a neurological syndrome in which despite being conscious patient is unable to communicate [4]. It is rarely reported in snake bite [5,7]. We report 4 children with LIS following snake bite.

### CASE-REPORTS

*Case 1:* A 2-year-old child presented with bleeding from ear, followed by altered sensorium and respiratory failure within two hours of initial complaints. At admission, child had respiratory arrest with severe bradycardia, absent peripheral and central pulses, and Glasgow coma scale (GCS) score of 3; pupils were fixed and dilated and doll's eye movements were absent. After initial resuscitation, he was noted to have some movement of right toe. Seven hours later, snake bite was suspected and polyvalent anti-snake venom (ASV) was administered. Thirty hours later, after receiving 25 vials, child had spontaneous respiration, movements of lower limbs and eye opening but pupils were still fixed and dilated. Pupillary reactions became normal on day three, doll's eye movement appeared a day later, and the child was discharged in premonitory condition on day ten.

*Case 2:* A ten-year-old boy presented with history of anxiety, pain abdomen and vomiting appearing acutely early morning, while sleeping on the floor of his cottage.