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Atypical hereditary spherocytosis phenotype associated with pseudohypokalaemia and a new variant in the band 3 protein

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SUMMARY

Red blood cell (RBC) membrane disorders are predominantly caused by mutations resulting in decreased RBC deformability and permeability. We present a family in which, the proband and his daughter presented with pseudohypokalaemia. Studies on the temperature dependence of pseudohypokalaemia suggested a maximum decrease in serum potassium when whole blood is stored at 37°C. Routine haematology suggested mild haemolysis with a hereditary spherocytosis phenotype. These two cases present a novel variant in temperature-dependent changes in potassium transport. A new variant was identified in the SLC4A1 gene which codes for band 3 protein (anion exchanger 1) in RBC membrane which may contribute to the phenotype. This is the first report of familial pseudohypokalaemia associated with changes in RBC membrane morphology. The clinical implications of pseudohypokalaemia are that it can lead to inappropriate investigation or treatment. However, many questions remain to be solved and other RBC membrane protein genes should be studied.

BACKGROUND

RBC membrane is composed of a fluid double layer of lipids in which approximately 20 major proteins and 850 minor proteins are embedded. The membrane is attached to the intracellular cytoskeleton by protein–protein and lipid–protein interactions. The structure is required for the RBC to maintain its shape and stability and deformability. Many transmembrane (TM) proteins have a transport function and a structural function when the intracytoplasmic domain interacts with cytoskeletal proteins. For example, band 3 both interacts with cytoskeletal proteins and is the red cell chloride/bicarbonate anion exchanger; in the tissues red cells take up CO_2 where it is converted to the bicarbonate ion, which leaves the cell in exchange for the chloride ion via band 3.[1]

Many families with red cell membrane disorders of varying severity have been described. Hereditary spherocytosis (HS) is a disorder that involves altered membrane structural organisation. In most cases of HS mutations are located in the following genes *ANK1, SPTB, SLC4A1, EBP42* and *SPTA1* which encode for Ankyrin, spectrin β-chain, band 3 protein (the red cell chloride/bicarbonate anion exchanger 1), protein 4.2 and spectrin α-chain. Mutations for other disorders hereditary elliptocytosis (HE) and hereditary pyropoikilocytosis (HPP) are in the *SPTA1* and *SPTB* gene and in the *EPB41* gene. The latter gene encodes protein 4.1.[1] HS, HE and HPP are phenotypically and genetically heterogeneous blood cell membrane disorders.[2 3] The presence of another RBC defect can enhance or reduce the phenotypic effects of HS, HE or HPP.[4 5]

Normal RBC has very low basal permeability (leak) to cations which is counteracted by Na, K-ATPase. The leak is temperature dependent. Red cell membrane disorders can also be secondary to altered membrane transport function. Hereditary stomatocytosis has several phenotypes associated with distinct genetic changes. The cryohydrosis phenotype which includes South-east Asian Ovalocytes results from mutations in the *SLC4A1* and the rare condition; stomatin-deficient cryohydrosis is caused by mutations in *SLC2A1*. Mutations in *RHAG* cause the highly leaky condition overhydrated stomatocytosis and mutations in *ABCB6* cause familial pseudohyperkalaemia (FP). All of the above are large multispanning membrane proteins. More recently mutations have been found in two RBC cation channels *PIEZO1* and *KCNN4* which result in dehydrated stomatocytosis. Changes in cation transport are a common factor in these disorders; however, these disorders show a wide heterogeneity in the degree of cation leak, temperature dependence of the leak and presenting symptoms.[6]

Measurement of the temperature dependence of the abnormal cation leak in red cell membrane disorders is a useful means of identifying leaky mutations. The temperature dependence of the abnormal leak differs in the steepness and minimum temperature of the cation leak than normal controls. Bruce *et al*[7] identified several different phenotypes associated with SLC4A1 mutations, including the cryohydrocytosis form of stomatocytosis, and HS associated with a large cation leak. Red cells from the affected pedigrees showed a deficiency of band 3 anion transport. The mutant RBCs have an increased permeability to cations though no study so far has shown that the cation leak is through the band 3 protein.

Red cells from FP patients exhibit a loss of K+ at low temperatures, <37°C mostly at 4°C. The temperature dependency of the cation leak is variable.[8–10] FP has been associated with HS and hereditary stomatocytosis.[11] Other authors describe the haematological abnormalities as negligible or as atypical HS.[1] The gene responsible for FP was identified as *ABCB6* which encodes the protein ABCB6, previously identified as a porphyrin transporter,

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which belongs to the family of ABC transporters with a binding cassette for ATP. The protein plays a role in heme synthesis. It is not clear whether the mutant ABCB6 protein can generate a cation leak pathway or secondarily change conformation of other proteins that dysregulate membrane cation permeability.[11 12]

We present a case that does not fit into the above categories. We describe a family previously classified as HS phenotype who presented in a novel form with familial pseudohypokalaemia. The patient and his daughter were recalled to hospital as an emergency for a repeat electrolyte measurement when a routine samples were found to show a low serum potassium. Repeat serum potassium measurements in an emergency department were within reference intervals. To our knowledge, this is the first case presentation of pseudohypokalaemia associated with the HS phenotype.

CASE PRESENTATION

The proband was a 57-year-old man (patient A). Multiple outpatient blood samples indicated hypokalaemia; this resulted in hospital admissions. On several occasions, he presented with outpatient serum potassium concentrations which were at times as low as 1.9 and 2 mmol/L. Hospital inpatient serum potassium values were, however, within reference range. He was taking omeprazole, ramipril for mild hypertension and ventolin for intermittent wheeze. He was referred by his community practitioner for investigation of a renal tubular defect as his 21-year-old daughter presented similarly with intermittent hypokalaemia (patient B) with serum potassium values of 2.8 and 3.3 mmol/L. Repeat serum potassium was within the reference range. She was being treated for vitamin B_{12} and folate deficiency, and taking contraceptives but was otherwise well. She had no symptoms of muscle weakness. Decrease in serum potassium was not associated with changes in average daily temperatures. A review of the medical records of the probands wife and son did not show episodes of hypokalaemia. The proband's wife was a type II diabetic and his son a type I diabetic. The wife and son were not investigated further. Written consent was obtained from both patients prior to this case presentation.

INVESTIGATIONS

The results of routine haematology and biochemistry studies of the proband (patient A) and his daughter (patient B) are given in in table 1. Magnesium, bicarbonate and chloride were within reference range, as were serum calcium, liver enzymes and serum cortisol. The proband's renin/aldosterone levels were measured following a change in hypertensive medication from ramipril to doxasozin for a period of 4 weeks. Both the proband and his daughter showed a slight increase in plasma renin levels. A Doppler scan of the proband's kidneys did not detect renal artery stenosis. Further haematological investigations showed a high serum bilirubin, reduced haptoglobin, increased MCV and increased reticulocytes, suggesting mild haemolytic anaemia. Direct antiglobulin tests were negative. The proband had been diagnosed with HS 25 years ago, with a blood film showing 'many spherocytes'. Blood film from the patient and his daughter suggested a spherocytic phenotype and spherocytes were 'noted' in the report (figure 1A,B). Eosin-5′-maleimide (Addenbrookes Hospital, UK) binding studies for both patients were below the reference range, consistent with the diagnosis of HS (table 1).

To confirm decrease in serum/plasma potassium with time, we used a screening method similar to one described previously.[13] In brief, blood from both patients and a control were drawn into either heparinised or non-heparinised tubes and incubated at 4°C, room temperature (23°C, RT) and 37°C for a maximum of 10 hours. Controls were women aged 68 and 69 years old with full blood count, renal function, bone studies and liver enzymes within reference range. Directly after venepuncture and at the stated time intervals either serum/plasma was separated and potassium concentration was measured using an ion selective electrode (Roche, UK). Haemolysis index was measured and showed no significant increase in haemolysis. Both patient and controls showed normal electrolyte concentrations at venepuncture. However, patient samples stored at RT and 37°C showed a progressive decrease in serum/plasma potassium at 3 hours. There were no significant differences in the decrease in plasma and serum potassium stored at RT and 37°C for patient A. There was a marked decrease in both serum and plasma potassium at 3 hours (figures 2A–D and 3A–C) in both the proband and his daughter on storage at RT and 37°C which was not observed in the controls. The mean decrease in the patient potassium (figure 4) at 3 hours was −19% and −31% compared with −0.03% and +4.3% ($\rho<0.03$) in the controls at RT and 37°C, respectively.

Next-generation sequencing was carried out on the Illumina Miseq (Molecular hematology, Oxford, UK) using a custom targeted panel which consisted of several genes associated with RBC membrane disorders: *ABCB7, ALAS2, ALDOA, ANK1, C15orf41, CDAN1, ENO1, EPB41, EPB42, G6PD, GATA1, GATA2, GCLC, GPI, GPX1, GSR, GSS, HK1, KIF23, KLF1, LPIN2, NT5C3A, PFKM, PGK1, PIEZO1, PKLR, RHAG, RPL5, RPL9, RPL11, RPL26, RPL27, RPL35A, RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPS27, RPS29, SBDS, SEC23B, SLC2A1, SLC4A1, SLC11A2, SLC25A38, SPTA1, SPTB, TMPRSS6, TPI1*. Common mutations were not detected. However, sequencing

Table 1 Summary of haematology and biochemistry results

Biochemistry	Patient A	Patient B	Haematology	Patient A	Patient B
Sodium (RR 138–146 mmol/L)	138	141	Haemoglobin (RR 135–180 g/L)	154	147
Potassium (RR 3.5–5.3 mmol/L)	4.1	3.5	WBC (RR 4.0–11 10*9/L)	8.3	6.3
Urea (RR 2.5–7.8 mmol/L)	6.5	4.2	Platelets (RR 150–400 10*9)	132	221
Creatinine (RR 62–106 µmol/L)	69	55	RBC (RR 4.5–6.5 10*12/L)	4.54	4.1
Chloride (RR 95–106 mmol/L)	97	102	HCT (0.4–0.54)	0.466	0.410
Bicarbonate (RR 22–29 mmol/L)	28	25	MCV (78–96 fl)	102.6	100
Total bilirubin (RR <21 µmol/L)	33	28	MCH (28–32 pg)	33.9	35.8
Magnesium (RR 0.7–1.6 mmol/L)	0.83	0.78	RDW (11%–16%)	20.5	14.5
Haptoglobin (RR 0.3–2.0 g/L)	0.1	<0.1	Reticulocytes (50–150 10*9/L)	345.4	266.6
Cortisol (RR 70–500 at 09:00, nmol/L)	365		EMA binding studies (NR <0.8)	0.8	0.65
Aldosterone (RR 90–700 pmol/L)	310	180			
Renin activity (RR 0.5–3.5 nmol/L/h)	3.9	4.6			
Urine sodium mmol/L	107	165			
Urine potassium mmol/L	40.8	60.1			
Urine pH	5.1	6.6			
Urine osmolality mOsm/kg	756	862			

EMA, eosin-5′-maleimide; HCT, hematocrit; HS, hereditary spherocytosis; MCH, mean cell hemoglobin; MCV, mean cell volume; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.

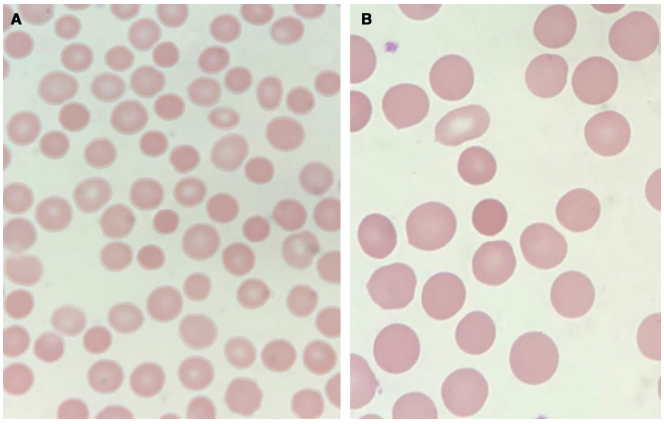

Figure 1 (A) Blood film (Giemsa stain) from proband (patient A) with spherocytes. (B) Blood film (Giemsa stain) from affected family member (patient B) with spherocytes.

of the *SLC4A1* identified both the proband and his daughter as heterozygous for an *SLC4A1* Trp496Leu variant. The variant is not listed in the Online Mendelian Inheritance in Man (OMIM) database (OMIM Entry -+109270 - SOLUTE CARRIER FAMILY 4 (ANION EXCHANGER), MEMBER 1; SLC4A1. HGVS classification SLC4A1 Heterozygous Paternal NM_000342.2: c.1487G>T,)

OUTCOME AND FOLLOW-UP

Although a rare finding, pseudohypokalaemia can lead to inappropriate treatment. Both patients are under the care of the general practitioners who will during follow-up health screens measure electrolytes within 5 min of phlebotomy.

DISCUSSION

Previous studies have indicated that exposure of blood to lower temperatures is associated with a rise in serum potassium concentration and high temperatures will result in a fall in serum potassium, even in healthy individuals. In one study, no significant change in serum potassium was observed at 20°C.[14] In FP, the severity of hyperkalaemia at low temperatures is higher than that found in controls.[15] In our family, the severity of hypokalaemia observed during storage of patient blood at RT and 37°C was increased compared with normal controls.

A detailed review on the crystal structure of band 3 protein has been published.[16] Mutation of the Trp492 or Trp496 within the transmembrane region4 (TM4) is predicted to cause band 3 to misfold. The two residues face outward from the same side of TM4 and are in close contact with N-terminal region of TM8.[17]

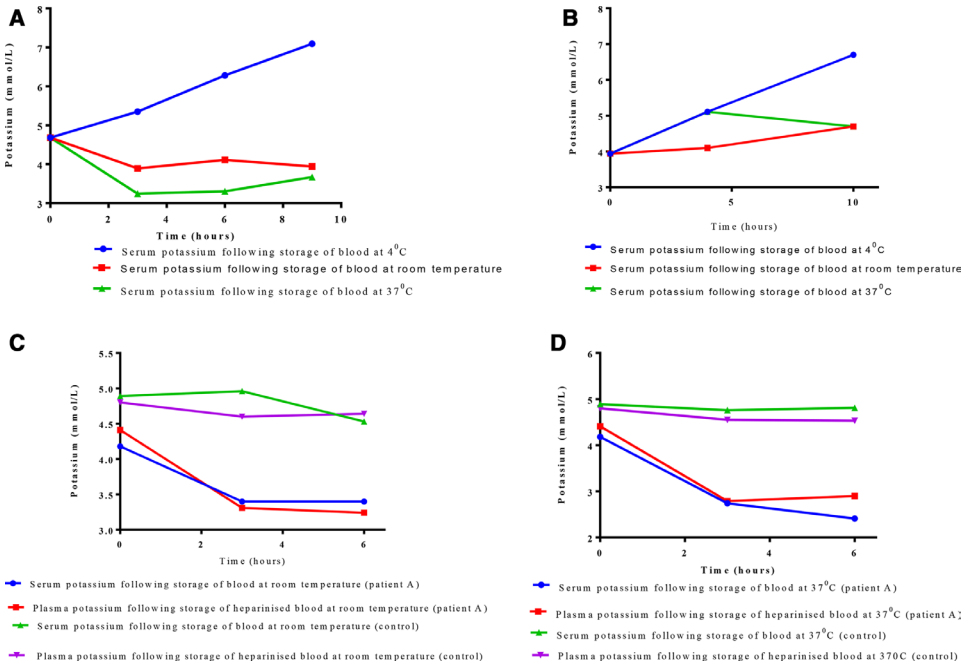

Figure 2 (A) Patient A: studies of changes serum potassium with storage: Serum potassium following storage of blood at 4°C. Serum potassium following storage of blood at room temperature. Serum potassium following storage of blood at 37°C. Decrease in serum potassium is more marked with increasing storage temperature. (B) Control on the same day as (B): studies of changes in serum potassium with storage. Serum potassium following storage of blood at 4°C. Serum potassium following storage of blood at room temperature. Serum potassium following storage of blood at 37°C. Control indicates slight increase in serum potassium with storage and temperature. (C) Comparison of serum and plasma potassium with storage of blood at room temperature: patient A and control. Serum potassium following storage of blood at room temperature (patient A). Plasma potassium following storage of heparinised blood at room temperature (patient A). Serum potassium following storage of blood at room temperature (control). Plasma potassium following storage of heparinised blood at room temperature (control). Effect of storage of blood at room temperature. Patient A shows a sharp decrease in both serum and plasma potassium with time, while the control serum and plasma potassium remain relatively stable. (D) Comparison of serum and plasma potassium with storage of blood at 37°C: patient A and control. Serum potassium following storage of blood at 37°C (patient A). Plasma potassium following storage of heparinised blood at 37°C (patient A). Serum potassium following storage of blood at 37°C (control). Plasma potassium following storage of heparinised blood at 37°C (control). Effect of storage of blood at 37°C. Patient A shows a sharp decrease in serum and plasma potassium with time, while the control serum and plasma potassium remain relatively stable.

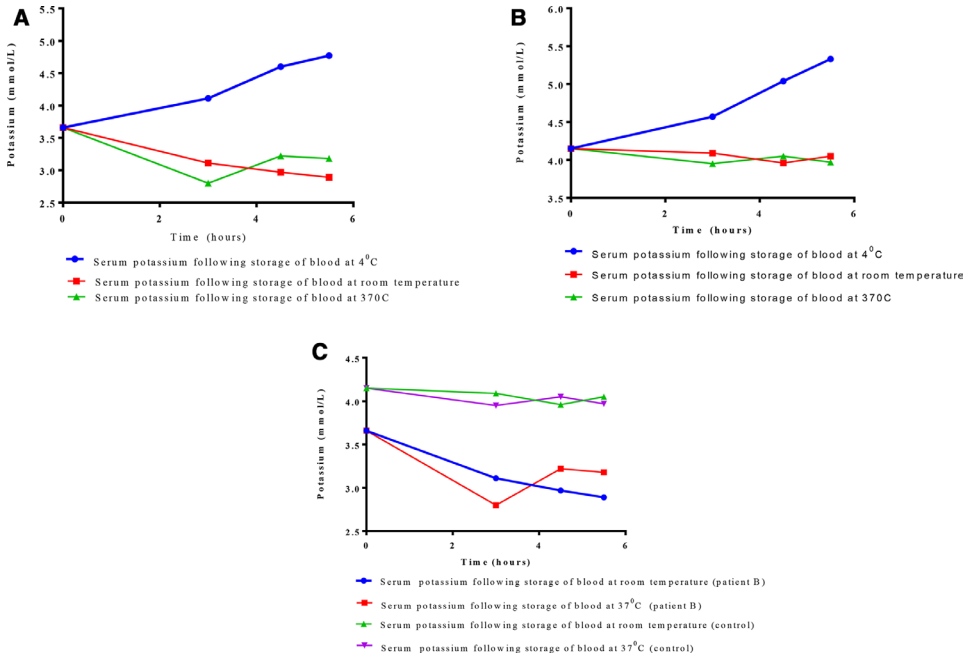

Figure 3 (A) Patient B: comparison of serum potassium with storage. Serum potassium following storage of blood at 4°C. Serum potassium following storage of blood at room temperature. Serum potassium following storage of blood at 37°C. Decrease in serum potassium is more marked with increasing storage temperature. (B) Control on same day: comparison of serum potassium with storage. Serum potassium following storage of blood at 4°C. Serum potassium following storage of blood at room temperature. Serum potassium following storage of blood at 37°C. Serum potassium remains stable at RT and 37°C. (C) Comparison of serum and plasma potassium with storage of blood at RT and 37°C: patient B and control (replot of graph 3a and c). Serum potassium following storage of blood at 37°C (patient B). Serum potassium following storage of blood at room temperature (patient B). Serum potassium following storage of blood at room temperature (control). Serum potassium following storage of blood at 37°C (control). Patient B serum potassium shows a marked decrease with temperature while control serum potassium remains relatively stable.

It is possible that replacement of the bulky Trp496 with a Leu residue as found in our patients can disrupt the packing of this region. Prediction of the consequences of these structural changes to the cation leak remains uncertain. Other mechanisms are that the mutant band 3 activates other endogenous transporters causing a cation imbalance. Consistent with this proposal is that the modelled three-dimensional structures of the mutant ABCB6 polypeptide predicted modest structural alterations of TM and cytosolic ATP binding domains.[5] It is likely that in vivo red cell homeostatic mechanisms maintain normokalaemia in the physiological state.

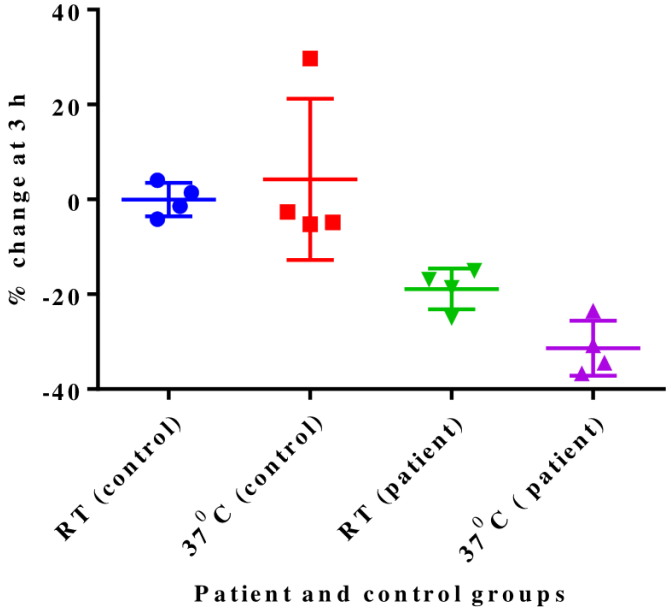

Figure 4 Percentage decrease in serum potassium at 3 hour at different temperatures compared in patient and control groups. RT, room temperature.

The human SLC4A1 encodes the kidney anion exchanger 1 (kAE1) which lacks the first 65 amino acids of AE1. Mutations that cause dRTA seldom affect red cell cation transport, though compound heterozygotes of distal renal tubular acidosis (dRTA) mutations and mutations that cause Southeast Asian ovalocytosis can exhibit dRTA and altered erythrocyte shape.[18] Neither the proband nor his daughter showed hypokalaemic hyperchloraemic acidosis, serum bicarbonate levels and urine pH were within the reference range. Both patients showed an increase in plasma renin activity, suggesting a possible renal involvement secondary to the *SLC4A1* variant.

There are a large number of transporters and channels in the RBC membrane which determine RBC volume and normal cell water content of the cell. There are several excellent reviews on this subject.[6 19] RBC has limited capacity to respond to alterations in monovalent cation content, and, if exceeded, cellular volume will change in parallel with the change in the total content of cations. In both our patients, MCV was increased, despite the presence of spherocytes suggesting an increase in RBC size. The major protein responsible for maintaining the high potassium, low sodium intracellular state is Na^+-K^+-ATPase which is an ATP-dependent pump that exchanges three sodium ions outwards for two potassium ions inwards. Other membrane ion transporters are Na^+-K^+-Cl^- cotransporters, K^+-Cl^- cotransporters, PIEZO1 a

mechanosensitive cation channel. Cation fluxes are disturbed in a group of inherited disorders, and these disorders show a wide heterogeneity in the severity of cation leaks and accompanying symptoms. Although the RBC membrane has been well studied, new insights have been reported into the proteins present and their role in RBC function. This raises the possibility that further studies into other membrane transport proteins will increase the understanding of this phenotype and segregate it into a distinct genetic background. Quantitation of red cell membrane proteins which include band 3 proteins, using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and comparison of red cell membrane proteins with individuals with normal FBC or unaffected family members will provide further evidence for the association between red cell membrane abnormality and the presenting phenotype.[20]

We present a family with a red cell membrane abnormality which results in an HS phenotype and pseudohypokalaemia. Clinically, the patients were asymptomatic and peripheral blood smears demonstrated HS and biochemistry suggested mild hyperbilirubinaemia with hemolytic anaemia. The true underlying molecular cause of this condition remains obscure, but it is suggested that a *SLC4A1* variant gene could contribute to the condition. The clinical implications of pseudohypokalaemia are that it can lead to inappropriate investigation or treatment.[5]

Learning points

- We report, to our knowledge for the first time, a variation of the hereditary spherocytosis phenotype with increased red blood cell MCV and pseudohypokalaemia.
- Severe hypokalaemia is a potentially life-threatening condition requiring immediate medical attention.
- Pseudohypokalaemia can be misleading and result in incorrect interpretation and patient mismanagement.
- Immediate recognition of pseudohypokalaemia and appropriate intervention can prevent misdiagnosis.
- The true underlying molecular cause of this condition remains obscure, but a possibility is that the *SLC4A1* missense mutation which predicts structural alterations of transmembrane domains could contribute to changes in cation permeability.

Contributors I was responsible for the conception and design of the case study; the analysis, and interpretation of data for the work as well as drafting the manuscript and its intellectual content.

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