

CASE REPORT

Open Access



A novel variant of SLC4A1 for hereditary spherocytosis in a Chinese family: a case report and systematic review

Jie Li^{1*}, Xiaozhi Wang², Na Zheng¹, Xiaoning Wang¹, Yan Liu¹ and Liying Xue^{2*} 

Abstract

Background: The incidence of hereditary spherocytosis (HS) is approximately 1:2000 in the western population, while it is much lower in the Chinese population. It is difficult to make a definite diagnosis due to the variable genotypic features and the lack of well-documented evidence for HS patients. Gene sequence examination is helpful for clear diagnosis.

Case presentation:: We presented the case of a 29-year-old male HS patient with skin yellowness, anorexia, and cholecystolithiasis as the first manifestations. Laboratory examination of the patient and his parents showed a mild reduction in hemoglobin and mean corpuscular hemoglobin concentration, increased reticulocytes, and promotion of indirect bilirubin in the patient and his father. Furthermore, small globular red blood cells with increased osmotic fragility were observed. In particular, the eosin-5'-maleimide binding test provided the strong evidence that band 3 protein was deleted in the erythrocyte membrane. Next-generation sequencing (NGS) and Sanger sequencing further demonstrated a heterozygous nonsense variant (exon16, c.G1985A: p.W662X) in SLC4A1, inherited from his father. Thus, the patient was diagnosed with HS, and then was effectively treated. After splenectomy, the anemia was relieved without any obvious unpleasant side effects.

Conclusion: We report an extremely rare case of HS in China that presented with hereditary hemolytic anemia with band 3 deletion resulting from a novel variant of SLC4A1, and systematically review a large number of related literatures. This study, therefore, significantly contributes to the literature on HS.

Keywords: Hereditary spherocytosis, SLC4A1, Band 3, Case report, Systematic review

Background

Hereditary spherocytosis (HS) refers to a group of heterogeneous inherited anemias. In the western population, it is the most common cause of hereditary hemolytic anemia, with an estimated incidence of 1:2,000 [12]; however, based on clinical reports, this condition seems less common in southeast Asian and African-American

populations [1]. In the Chinese population, the prevalence is approximately 1 in 100,000 people [3]. Morphologically, HS is characterized by the presence of spherocytes in peripheral blood smear, and is generally due to variants in one of the five genes (SPTA1, SPTB, ANK1, SLC4A1, and EBP42), encoding α -spectrin, β -spectrin, ankyrin, band 3 (anion exchanger 1, AE1), and protein 4.2, respectively [4]. Among these genes, ANK1 and SPTB variants are the most frequent causes of HS, followed by variants in SLC4A1 (15%) in Northern European populations [5]. However, the Asian population showed a lower rate of variant in SLC4A1, with < 13.5% in the Chinese population [67–11] and only 4.2%

*Correspondence: doclijie@163.com; xueliying123@163.com

¹ Department of Hematology, Hebei General Hospital, 348 West Heping Road, 050000 Shijiazhuang, China

² Laboratory of Pathology, Hebei Medical University, No. 361, Zhongshan Eastern Road, 050000 Shijiazhuang, China



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

in the Indian population [12]. Although relevant studies on HS in the Chinese population have been published in the last 2 years, in some cases, it is difficult to make a definite diagnosis due to the variable genotypic and phenotypic features of HS and the lack of well-documented evidence for HS patients. Timely diagnosis and therapy will help decrease complications of biliary tract disease, such as biliary obstruction with pancreatitis, cholecystitis, and cholangitis, and contribute to improving patients' quality of life [21315]. Thus, accurate detection of known or new variant sites associated with HS is important in understanding this genetic disease.

Herein, we describe a case of HS in a 29-year-old man caused by a novel stopgain variant (c.G1985A) in SLC4A1 exon 16, inherited from his father. Notably, the patient exhibited more severe hemolytic anemia than his father and presented with splenomegaly, cholelithiasis, and kidney disease. Based on genetic screening for hereditary diseases of the hemopoietic system and immunodeficiency diseases in selected family members of the patient, we concluded that a new variant in SLC4A1 caused the phenotypic deficiency of band 3 (p.W662X) in this family, subsequently leading to the onset of HS. The patient provided written informed consent for the publication of this study, which was approved by the Ethics Committee of Hebei General Hospital, Shijiazhuang, China.

Case presentation

A 29-year-old man was admitted to Hebei General Hospital (Shijiazhuang) on July 7, 2020, due to skin yellowness, anorexia, nausea, and vomiting after satiety, occasional abdominal distension, and dizziness. Four months before presenting to our hospital, the patient was initially diagnosed with cholecystolithiasis because of similar symptoms and abdominal ultrasound results (splenomegaly and bile duct neck stones) and did not receive treatment at a county hospital. The patient was subsequently admitted to our hospital with complaints of weight loss and unrelieved symptoms resulting from cholecystolithiasis. More than 10 years ago, the patient's father showed mild anemia, splenomegaly and elevated bilirubin without a clear diagnosis and further treatment. On admission, the estimation of the complete blood count, a mild reduction in hemoglobin and mean corpuscular hemoglobin concentration was observed in the blood of the patient and his father (Table 1). Laboratory examination revealed increased number of reticulocytes and increased levels of total bilirubin and indirect bilirubin (Table 1). However, the patient showed negative results on immunofluorescence diagnosis and Coombs' test. Peripheral blood smears for the patient and his parents showed different forms and sizes of mature red blood cell (RBC), with small globular RBC in the patient

(2.4%) and his father (Fig. 1). The osmotic fragility test for the patient showed that a significant increase in RBC osmotic fragility (Table 2).

Furthermore, the eosin-5'-maleimide (EMA) binding test using flow cytometry (FCM) showed decreased fluorescence of EMA-labeled RBCs, with a mean fluorescence intensity of only 30.33% (Fig. 2), providing strong evidence that the band 3 protein is deleted in the RBC membrane. Based on the father's medical history of anemia and splenomegaly, DNA from the patient and his parents were screened for nearly 700 genes related to hereditary blood and immunodeficiency diseases (SPTB, SPTA1, EPB41, EPB42, ANK1, SCL4A1, ALAS2, SFXN4, TET2, HSPA9, HBA1, MTR, MMAB, etc.) using the next-generation sequencing (NGS). The results showed a heterozygous nonsense variant (NM_000342: exon16, c.G1985A: p.W662X) in SLC4A1. Sanger sequence further demonstrated that this variant was inherited from his father, but not from his mother (Fig. 3). According to the Mutational Database, including 1000 Genomes Project, dbSNP, ClinVar, ESP6500, ExAc, Ensembl, HGMD, and UCSC, this variant has not been reported previously. Meanwhile, it was predicted to be pathogenic variant (PVS1 + PS1 + PM2) based on the American College of Medical Genetics and Genomics (ACMG) standards and guidelines.

Additionally, abdominal ultrasound showed fatty liver, hepatomegaly, multiple gallstones, splenomegaly, and splenic vein widening. Abdominal and pelvic computed tomography (CT) further demonstrated multiple gallstones, splenomegaly, and left renal calculi (Fig. 4).

Based on the prominent symptoms, laboratory results, and DNA screening, the patient was diagnosed with HS, gallbladder stone, and left kidney stone. He began treatment with folic acid and mecobalamin. After splenectomy, the anemia was relieved without any obvious unpleasant side effects. And no occurrence of anemia was observed in the follow-up period.

Discussion and conclusion

The prevalence of HS is relatively high in North European countries but is much lower in the Asian population. A survey of hereditary hemolytic anemia in South Korea showed 71.3% of RBC membranopathies [16], while there has been no investigation on such a large number of cases due to the sporadic nature in China. Clinical data from the Changhai Hospital showed that of the hereditary hemolysis cases, 42.56% were membranopathies [17]. However, with the clinical application of gene sequencing, the number of reported cases of HS has significantly increased [18]. In the past 10 years, nearly half of the total HS cases have been reported, with 71% diagnosed at university hospitals [18]. Owing to the lack of accurate data

Table 1 Laboratory investigations

Investigations	On admission	Father	Mother
WBC (mm ³)	3630	4660	4280
Neutrophils (%)	57.3%	51.3%	47.1%
Lymphocytes (%)	37.2%	43.8%	47.7%
Monocytes (%)	4.1%	3.6%	4.2%
Eosinophils (%)	1.1%	1.1%	0.5%
Basophils (%)	0.3%	0.2%	0.5%
Erythrocyte (mm ³)	3,280,000	3,630,000	4,390,000
Hemoglobin (g/dl)	10.9	11.8	13.5
Platelets count (mm ³)	189,000	187,000	227,000
Prothrombin time (seconds)	10.8	11.6	9.9
Reticulocyte (mm ³)	466,100	410,900	52,700
Reticulocyte (%)	14.21%	11.32%	1.20%
Activated partial thromboplastin time (seconds)	25.2	28.5	27.1
Serum folate (ng/mL)	3.09	4.12	8.36
vitaminB-12 (pg/mL)	615.2	755.2	554.3
Serum ferritin (ng/mL)	693.4	525.7	32.6
Serum iron (μmol/L)	14.9	20.6	28.7
Total iron-binding capacity (μmol/L)	44.8	42.5	55.2
Transferrin (mg/dL)	196.4	183.3	262.8
Unasaturated iron binding force (μmol/L)	29.9	25.3	43.7
Coombs' test	Negative	Negative	Negative
Blood urea (mmol/L)	6.8	7.5	8.4
Serum creatinine (μmol/L)	85.2	66.3	58.7
Alanine aminotransferase (U/L)	30.6	27.8	25.5
Aspartate aminotransferase (U/L)	17.8	16.5	23.7
Total bilirubin (μmol/L)	111.5	133.0	13.8
Direct bilirubin (μmol/L)	12.3	13.0	3.8
Indirect bilirubin (μmol/L)	99.2	120.0	10.0
HBsAg	Negative	Negative	Negative
HBsAb	Negative	Negative	Negative
HCV-Ab	Negative	Negative	Negative
Anti-nuclear antibody	Negative	Negative	Negative

on the incidence of and a detailed study on HS, its early diagnosis remains unclear in the Chinese population. As HS is caused by variants in different genes, its clinical manifestations vary widely, ranging from asymptomatic hemolysis to transfusion dependence. Therefore, it is difficult to obtain the correct diagnosis and to provide early treatment through traditional examinations. Sequence analysis of genetic exons contributes to early diagnosis and understanding of the characteristics of the variants.

Physiologically, in the RBC membrane, an enormous number of transporters and channels determine RBC volume and intracellular water content. There are five causative genes of HS: SPTB, SPTA1, ANK1, SLC4A1, and EPB42, which encode the erythrocyte membrane proteins β -spectrin, α -spectrin, ankyrin 1, band 3, and

protein 4.2, respectively. Among these, the tetramer of spectrin forms a dense network, lining the inner surface of the lipid bilayer in the RBC membrane, while ankyrin-1 provides the main membrane binding site for the spectrin-based membrane skeleton and links β -spectrin to band-3 [219]. These skeleton proteins provide RBCs with deformability and undergo substantial distortion without fragmentation during microcirculation [1920]. Therefore, protein defects caused by gene variants result in decreased deformability, increased osmotic fragility, and premature destruction in the spleen. It has been reported that 75% of HS cases are associated with dominant inheritance and 25% are associated with non-dominant and recessive inheritance [52122, 23]. In Northern European populations, variants in ANK1 (50–60%) are the most

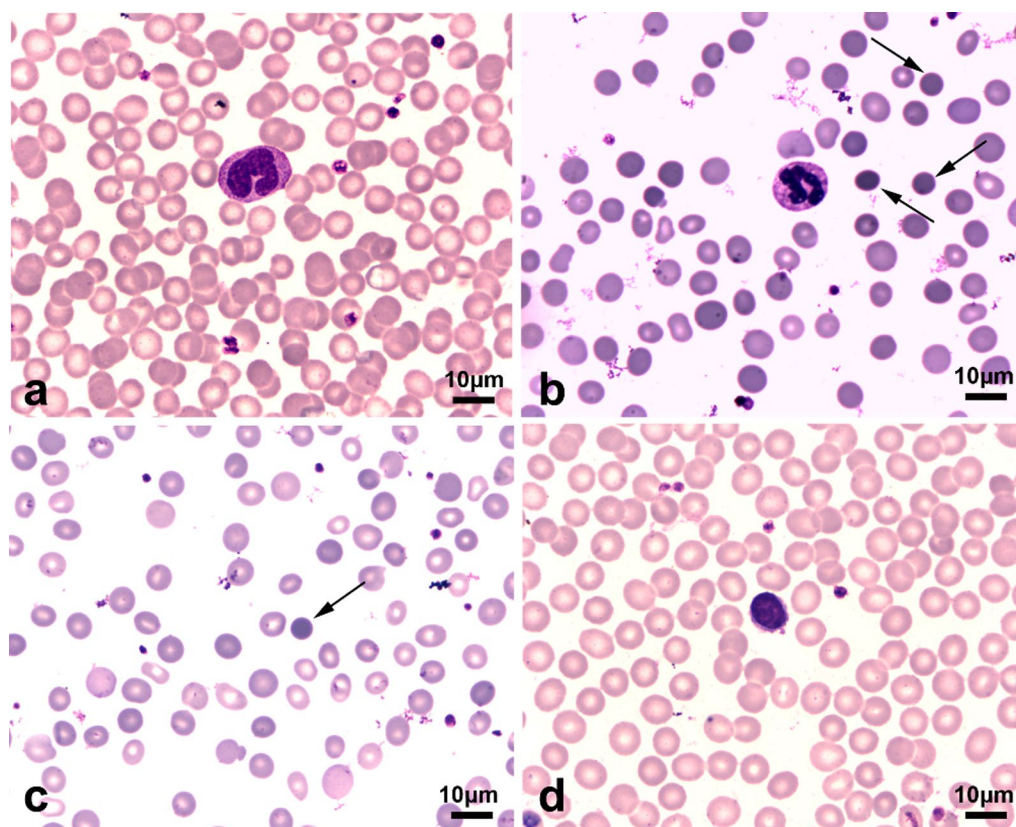


Fig. 1 Peripheral blood smears of the patient, his parents and healthy donor. Peripheral blood smears were detected on an optical microscope (Olympus BX53, Shinjuku, Tokyo, Japan) with a cell medical image system (CMIS-2011). **a** Peripheral blood smears of the healthy donor. **b** Peripheral blood smears of the patient showed multiple spherocytes (black arrows) lacking central pallor. **c** Peripheral blood smears of his father showed many spherocytes (black arrows). **d** Peripheral blood smears of his mother. ($\times 400$)

Table 2 The results of Hemolysis test

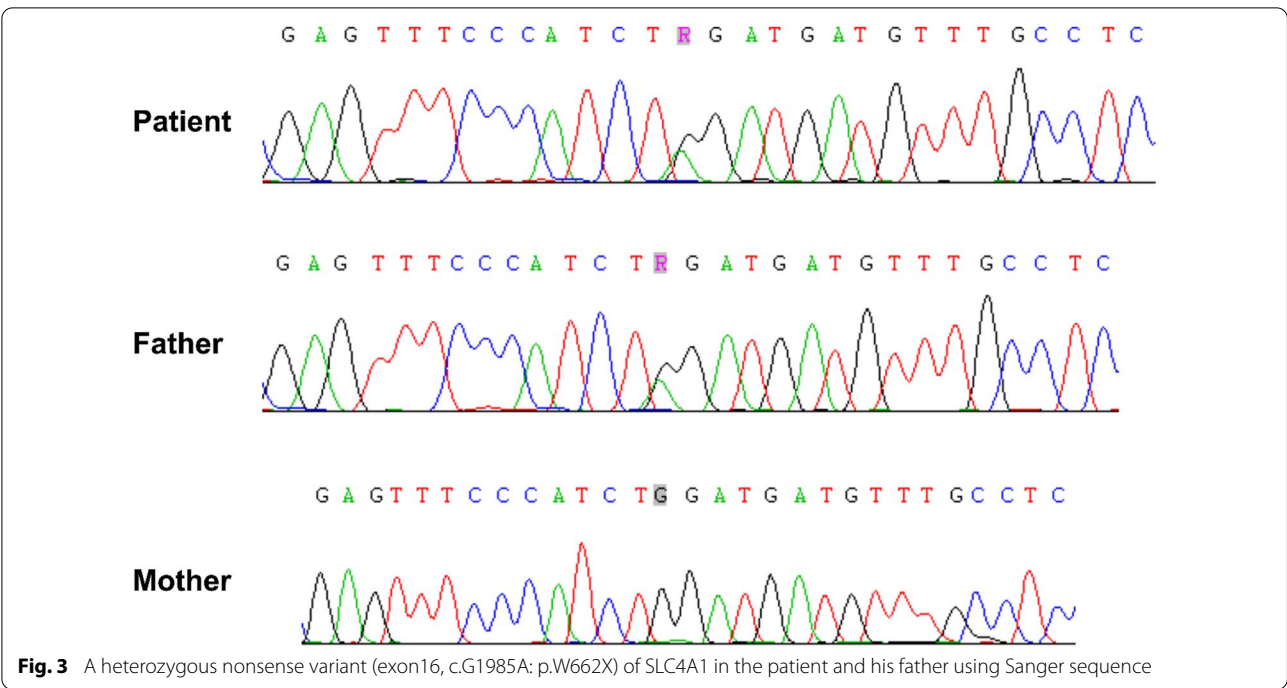
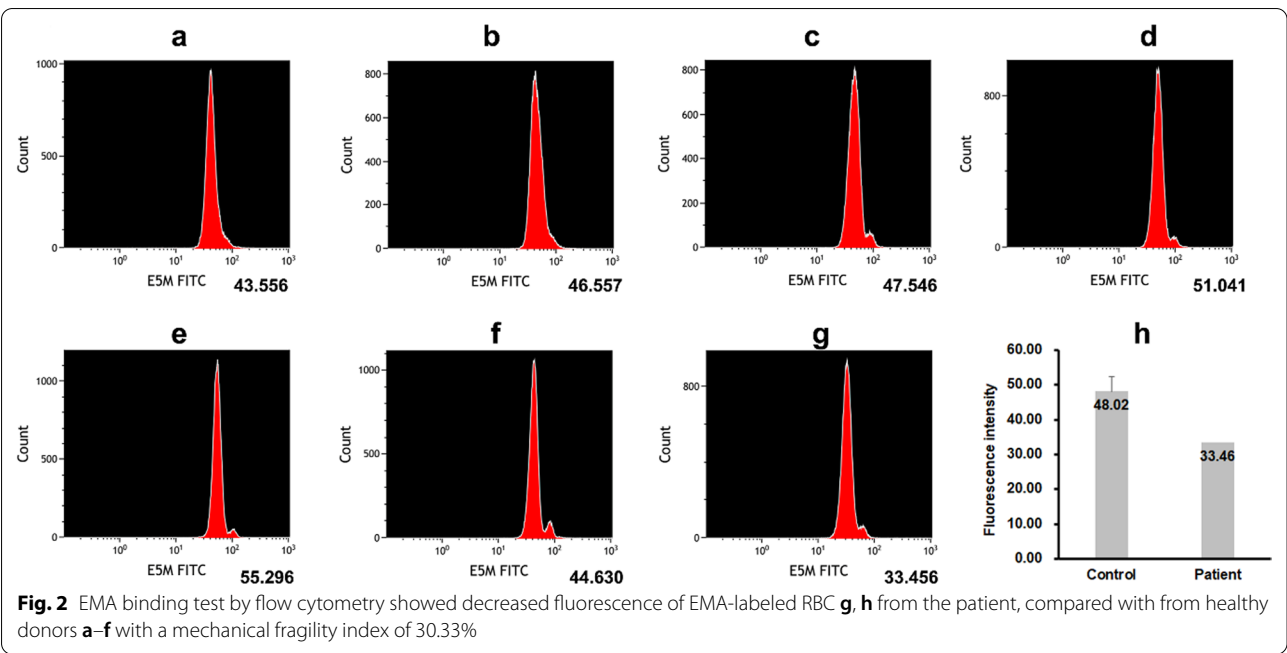
Investigations	On admission	Father	Mother	Reference values
Acidified glycerol lysis test (AGLT)	45s↓	43s↓	330s	> 290s
Osmotic fragility test (the beginning hemolysis)	0.56%↑	0.52%↑	0.45%	(0.44–0.48)%
Osmotic fragility test (the complete hemolysis)	0.48%↑	0.43%↑	0.32%	(0.28–0.36)%
Sucrose hypertonic test cold hemolysis (SHTCL)	24.3%↑	22.6%↑	10.8%	(0–16.9)%
Erythrocyte incubation osmotic fragility test	0.62%↑	0.61%↑	0.47%	(0.44–0.60)%

The down (↓) and up (↑) arrows represent abnormal value

frequent cause of HS, followed by variants in the SPTB or SPTA1 gene (20%) and in the SLC4A1 gene (15%) [5]. In 25 Korean patients, variants in ANK1 (52%) or SPTB (48%) were genetically reported to be the cause of HS [24], while heterozygous variants in ANK1 were found in 31% of Japanese HS patients [25]. A study of 73 Indian families (113 patients) with HS found variants in ANK1 (53.2%), SPTB (36.2%), and SLC4A1 (4.2%) [12]. The five most recent studies reported in the Chinese population showed incidences of 44.7–66.7% for ANK1 variant,

33.3–45.7% for SPTB variant, and <13.5% for SLC4A1 variant [67–11]. Compared to European countries, Asian countries show a lower rate of variants in SLC4A1, which are predominantly inherited.

In the present study, the patient presented with typical manifestations, including jaundice, anorexia, occasional abdominal distension, and dizziness. Laboratory examination showed an increased number of reticulocytes and increased levels of total bilirubin and indirect bilirubin, suggestive of RBC damage, compensatory erythrocytosis,



and hemolysis. However, the negative results of immunofluorescence diagnosis and Coombs' test excluded the possibility of paroxysmal nocturnal hemoglobinuria and autoimmune hemolytic anemia. Peripheral blood smears showed small globular RBC, and osmotic fragility tests showed increased osmotic fragility. Abdominal and pelvic CT further demonstrated multiple gallstones,

splenomegaly, and left renal calculi. Subsequently, EMACF provided strong evidence that the band 3 protein was deleted in the erythrocyte membrane. Based on these results, the patient was diagnosed with HS. To clarify HS diagnosis and genetic variant, WES for approximately 700 genes associated with hereditary diseases of the blood and immune system was performed on samples

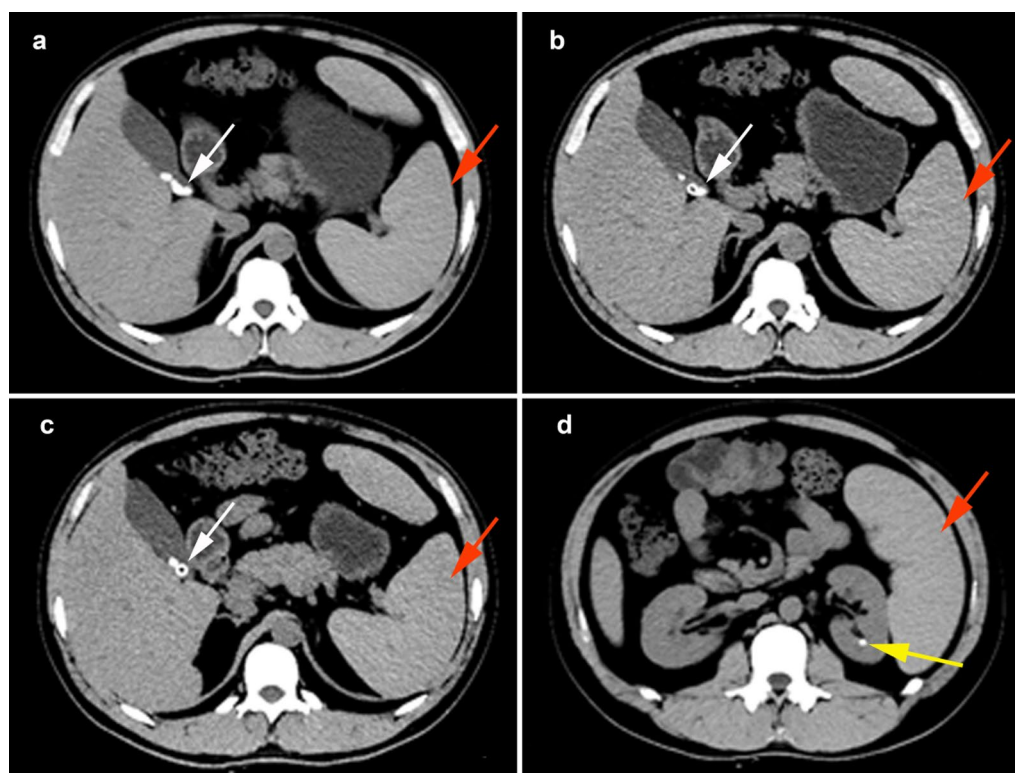


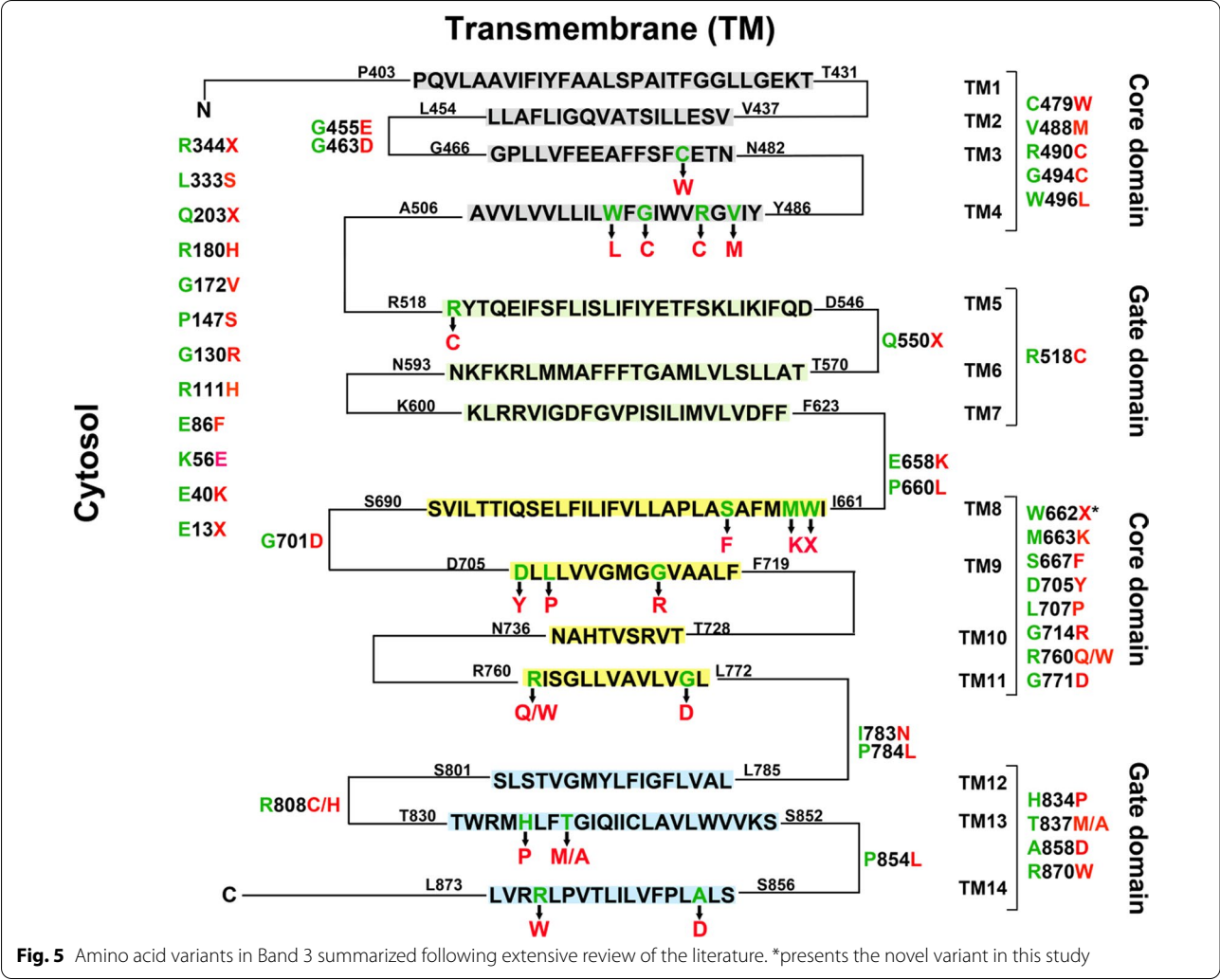
Fig. 4 Abdominal and pelvic CT scans showed multiple gallstones (white arrows in **a**, **b** and **c**), splenomegaly (red arrows in **a**, **b**, **c** and **d**) and left renal calculi (yellow arrow in **d**)

from the patient and his father. The results demonstrated a heterozygous stopgain variant in SLC4A1 exon16 (c.G1985A; p.W662X).

SLC4A1, consisting of 20 exons, encodes a 911 amino acid protein, Band-3 (referred to as NM_000342, NP_000333.1). Erythrocyte band 3 is a major membrane protein, with 1.2 million copies per cell. Functionally, it includes two major domains: (1) an N-terminal cytosolic domain (cdAE1), providing attachment sites for the skeleton (ankyrin 1, protein 4.1, adducin2, and protein 4.2), glycolytic enzymes, and deoxyhemoglobin [2627], and (2) a C-terminal membrane domain (mdAE1), which forms the anion-exchange channel and aids carbon dioxide transport through the exchange of chloride and bicarbonate ions [192829]. The mdAE1 of Band 3 consists of 14 transmembrane (TM) segments with the N- and C-termini facing the cytosol [30]. Among these segments, a core domain (TM1-4 and TM 8–11) provides anion-binding sites and a gate domain (TM5-7 and TM12-14) contains lysine residues crosslinked by some organic anions [30]. The membrane domain of Band 3, responsible for the transport function, is completely functional in the absence of the cytosolic domain [31].

Variants in SLC4A1 have been linked to four human diseases, including HS, Southeast Asian ovalocytosis (SAO), and hereditary stomatocytosis (HSt) [32], distal renal tubular acidosis (dRTA) [3334, 35]. SAO mutates frequently via the deletion of nine amino acids (Ala400-Ala408) on the cytosolic boundary region of TM1 [30], while many HSt variants cluster in or around TM10 on the cytoplasmic half of the core domain. For HS, variants in SLC4A1 are thought to occur throughout the sequence, including both the membrane and cytosolic domains. Although approximately one-third of the variants were reported to likely affect the processing of SLC4A1 pre-mRNA [1], variants in HS are common in the Band 3 membrane domain. Following extensive review of the literature, we summarized most of the amino acid variants in Band 3 (Fig. 5) [1693637–54]. To date, approximately 70% of the variants seem to occur in the membrane domain, more frequently than in the core domain.

In the case presented here, NGS results demonstrated a heterozygous variant (exon16, c.G1985A: p.W662X) in Band 3. Trp662, located near the beginning of TM8 (1661-S690), faces the terminal region of TM3. It has



been reported that tryptophan residues in Band 3 play key roles in energy transfer. For example, Trp848, located on the extracellular end of TM13, may be the predominant tryptophan residue responsible for energy transfer [20]. Variant of Trp492 or Trp496 within TM4 and in close contact with the N-terminal region of TM8 causes Band 3 to misfold. Meanwhile, the variant of Trp648, Trp662, or Trp723 to Ala has the same effect. In this study, the variant (c.1985G>A) in SLC4A1 (inherited from the father) caused the conversion of TGG (Trp) to a stop codon TAG, resulting in the loss of Band 3. This compromised the stability of the cytoskeleton in the RBC membrane and induced the onset of HS in the patient.

In summary, we report an extremely rare case of HS in a Chinese population that presented hereditary hemolytic anemia with the deletion of band 3 resulting from a novel variant (exon16, c.G1985A: p.W662X). Because

of the lack of a registration system for HS in China, the exact rate of its incidence and genotypic and phenotypic features of HS patients carrying variants in SLC4A1 remain unknown. Identifying potential genetic causes is helpful in understanding the correlation between genotype and phenotype. This study thus makes a significant contribution to the literature on HS.

Abbreviations
HS: Hereditary spherocytosis; AE1: Anion exchanger 1; RBC: Red blood cell; FCM: Flow cytometry; EMA: Eosin-5'-maleimide; WES: Whole exome sequencing; CT: Computed tomography; TM: Transmembrane; SAO: Southeast Asian ovalocytosis; HS: Hereditary stomatocytosis; dRTA: Distal renal tubular acidosis; WBC: White blood cell; AGLT: Acidified glycerol lysis test; SHTCL: Sucrose hypertonic test cold hemolysis; NGS: Next-generation sequencing.

Acknowledgements
Not Applicable.

Author contributions

JL, XW1, NZ, XW2 and YL collected, verified and interpreted patient information, JL and LX drafted the manuscript. JL, XW1, NZ, XW2 and YL diagnosed and treated the patient. JL and LX designed the research, and critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

Funding

This research was funded by the Natural Science Foundation of Hebei Province (Grant no. H2017307024), the Project of Financial Department of Hebei Province (Grant no. 202013) and the Project of Health Department of Hebei Province (Grant no. 20170030). The funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials

The NGS data generated and/or analyzed during this study are available in the NCBI Sequence Read Archive (SRA) repository (Accession Number: SRR20046343, SRR20046344, SRR20046345).

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Hebei General Hospital (Shijiazhuang, China). Written informed consent to participate was obtained from the patient, the patient's parents and the healthy donor.

Consent for publication

Written informed consent for publication of identifying images or other personal or clinical details was obtained from all of the participants.

Competing interests

The authors declare that they have no competing interests.

Received: 28 September 2021 Accepted: 14 November 2022

Published online: 03 December 2022

References

1. Van Zwieten R, François JJ, Van Leeuwen K, Van Wesel AC, Van Bruggen R, Van Solinge WW, et al. Hereditary spherocytosis due to band 3 deficiency: 15 novel mutations in SLC4A1. *Am J Hematol*. 2013;88(2):159–60. <https://doi.org/10.1002/ajh.23363>.
2. Perrotta S, Gallagher PG, Mohandas N. Hereditary spherocytosis. *Lancet*. 2008;372(9647):1411–26. [https://doi.org/10.1016/S0140-6736\(08\)61588-3](https://doi.org/10.1016/S0140-6736(08)61588-3).
3. Wang C, Cui Y, Li Y, Liu X, Han J. A systematic review of hereditary spherocytosis reported in Chinese biomedical journals from 1978 to 2013 and estimation of the prevalence of the disease using a disease model. *Intractable Rare Dis Res*. 2015;4(2):76–81. <https://doi.org/10.5582/irdr.2015.01002>.
4. Farias MG. Advances in laboratory diagnosis of hereditary spherocytosis. *Clin Chem Lab Med*. 2017;55(7):944–8. <https://doi.org/10.1515/cclm-2016-0738>.
5. An X, Mohandas N. Disorders of red cell membrane. *Br J Haematol*. 2008;141(3):367–75. <https://doi.org/10.1111/j.1365-2141.2008.07091.x>.
6. Wang R, Yang S, Xu M, Huang J, Liu H, Gu W, Zhang X. Exome sequencing confirms molecular diagnoses in 38 Chinese families with hereditary spherocytosis. *Sci China Life Sci*. 2018;61(8):947–53. <https://doi.org/10.1007/s11427-017-9232-6>.
7. Peng GX, Yang WR, Zhao X, Jin LP, Zhang L, Zhou K, et al. The characteristic of hereditary spherocytosis related gene mutation in 37 Chinese hereditary spherocytosis patients. *Zhonghua Xue Ye Xue Za Zhi (Chinese)*. 2018;39(11):898–903. <https://doi.org/10.3760/cma.j.issn.0253-2727.2018.11.005>.
8. Wu C, Xiong T, Xu Z, Zhan C, Chen F, Ye Y, et al. Preliminary study on the clinical and genetic characteristics of hereditary spherocytosis in 15 Chinese children. *Front Genet*. 2021;12:652376. <https://doi.org/10.3389/fgene.2021.652376>.
9. Xie F, Lei L, Cai B, Gan L, Gao Y, Liu X, et al. Clinical manifestation and phenotypic analysis of novel gene mutation in 28 Chinese children with hereditary spherocytosis. *Mol Genet Genomic Med*. 2021. <https://doi.org/10.1002/mgg3.1577>.
10. Qin L, Nie Y, Zhang H, Chen L, Zhang D, Lin Y, et al. Identification of new mutations in patients with hereditary spherocytosis by next-generation sequencing. *J Hum Genet*. 2020;65(4):427–34. <https://doi.org/10.1038/s10038-020-0724-z>.
11. Wang X, Zhang A, Huang M, Chen L, Qun H, Lu Y, et al. Genetic and clinical characteristics of patients With hereditary spherocytosis in Hubei Province of China. *Front Genet*. 2020;11:953. <https://doi.org/10.3389/fgene.2020.00953>.
12. Aggarwal A, Jamwal M, Sharma P, Sachdeva MUS, Bansal D, Malhotra P, et al. Deciphering molecular heterogeneity of Indian families with hereditary spherocytosis using targeted next-generation sequencing: first South Asian study. *Br J Haematol*. 2020;188(5):784–95. <https://doi.org/10.1111/bjh.16244>.
13. Da Costa L, Suner L, Galimand J, Bonnel A, Pascreau T, Couque N, et al. Diagnostic tool for red blood cell membrane disorders: assessment of a new generation ektacytometer. *Blood Cells Mol Dis*. 2016;56(1):9–22. <https://doi.org/10.1016/j.bcmd.2015.09.001>.
14. Bolton-Maggs PH, Langer JC, Iolascon A, Tittensor P, King MJ. General haematology task force of the British committee for standards in haematology. Guidelines for the diagnosis and management of hereditary spherocytosis–2011 update. *Br J Haematol*. 2012;156(1):37–49. <https://doi.org/10.1111/j.1365-2141.2011.08921.x>.
15. Manciu S, Matei E, Trandafir B. Hereditary spherocytosis-diagnosis, surgical treatment and outcomes. A literature review. *Chirurgia*. 2017;112(2):110–6. <https://doi.org/10.21614/chirurgia.112.2.110>.
16. Shim YJ, Jung HL, Shin HY, Kang HJ, Choi JY, Hah JO, et al. Epidemiological study of hereditary hemolytic anemia in the Korean pediatric population during 1997–2016: a nationwide retrospective cohort study. *J Korean Med Sci*. 2020;35(33):e279. DOI:10.3346/jkms.2020.35.e279.
17. Li J, Huang Z, Xu Y, Zhou H, Han F, Gong S, Wan S. Compound heterozygote factor and clinical significance of hemolysis system analysis in the diagnosis of congenital hemolytic anemia: etiological analysis of 506 cases of anemia and jaundice. *J Clin Hematol*. 2005;18(4):204–6 (in Chinese).
18. Wang C, Cui Y, Li Y, Liu X, Han J. A systematic review of hereditary spherocytosis reported in Chinese biomedical journals from 1978 to 2013 and estimation of the prevalence of the disease using a disease model. *Intract Rare Dis Res*. 2015;4(2):76–81. <https://doi.org/10.5582/irdr.2015.01002>.
19. Lux SE 4th. Anatomy of the red cell membrane skeleton: unanswered questions. *Blood*. 2016;127(2):187–99. <https://doi.org/10.1182/blood-2014-12-512772>.
20. Van Dort HM, Knowles DW, Chasis JA, Lee G, Mohandas N, Low PS. Analysis of integral membrane protein contributions to the deformability and stability of the human erythrocyte membrane. *J Biol Chem*. 2001;276(50):46968–74. <https://doi.org/10.1074/jbc.M107855200>.
21. Andolfo I, Russo R, Gambale A, Iolascon A. New insights on hereditary erythrocyte membrane defects. *Haematologica*. 2016;101(11):1284–94. <https://doi.org/10.3324/haematol.2016.142463>.
22. Narla J, Mohandas N. Red cell membrane disorders. *Int J Lab Hematol*. 2017;39(Suppl 1):47–52. <https://doi.org/10.1111/ijlh.12657>.
23. Iolascon A, Andolfo I, Russo R. Advances in understanding the pathogenesis of red cell membrane disorders. *Br J Haematol*. 2019;187(1):13–24. <https://doi.org/10.1111/bjh.16126>.
24. Park J, Jeong DC, Yoo J, Jang W, Chae H, Kim J, et al. Mutational characteristics of ANK1 and SPTB genes in hereditary spherocytosis. *Clin Genet*. 2016;90(1):69–78. <https://doi.org/10.1111/cge.12749>.
25. Nakanishi H, Kanzaki A, Yawata A, Yamada O, Yawata Y. Ankyrin gene mutations in Japanese patients with hereditary spherocytosis. *Int J Hematol*. 2001;73(1):54–63. <https://doi.org/10.1007/BF02981903>.
26. Campanella ME, Chu H, Low PS. Assembly and regulation of a glycolytic enzyme complex on the human erythrocyte membrane. *Proc Natl Acad Sci USA*. 2005;102(7):2402–7. <https://doi.org/10.1073/pnas.0409741102>.
27. Chu H, Low PS. Mapping of glycolytic enzyme-binding sites on human erythrocyte band 3. *Biochem J*. 2006;400(1):143–51. <https://doi.org/10.1042/BJ20060792>.
28. Lux SE, John KM, Kopito RR, Lodish HF. Cloning and characterization of band 3, the human erythrocyte anion-exchange protein (AE1). *Proc Natl*

- Acad Sci USA. 1989;86(23):9089–93. <https://doi.org/10.1073/pnas.86.23.9089>.
29. 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(7):1693–707. <https://doi.org/10.1172/JCI119694>.
 30. Reithmeier RA, Casey JR, Kalli AC, Sansom MS, Alguet Y, Iwata S. Band 3, the human red cell chloride/bicarbonate anion exchanger (AE1, SLC4A1), in a structural context. *Biochim Biophys Acta*. 2016;1858(7 Pt A):1507–32. <https://doi.org/10.1016/j.bbame.2016.03.030>.
 31. Shnitsar V, Li J, Li X, Calmettes C, Basu A, Casey JR, Moraes TF, Reithmeier RA. A substrate access tunnel in the cytosolic domain is not an essential feature of the solute carrier 4 (SLC4) family of bicarbonate transporters. *J Biol Chem*. 2013;288(47):33848–60. <https://doi.org/10.1074/jbc.M113.511865>.
 32. Barneaud-Rocca D, Pellissier B, Borgese F, Guizouarn H. Band 3 missense mutations and stomatocytosis: insight into the molecular mechanism responsible for monovalent cation leak. *Int J Cell Biol*. 2011;2011:136802. doi: 10.1155/2011/136802.
 33. 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(2):49–62. <https://doi.org/10.4172/1747-0862.1000013>.
 34. Yenchitsomanus PT, Sawasdee N, Paemanee A, Keskanokwong T, Vasuvat-takul S, Bejrachandra S, et al. Anion exchanger 1 mutations associated with distal renal tubular acidosis in the Thai population. *J Hum Genet*. 2003;48(9):451–6. <https://doi.org/10.1007/s10038-003-0059-6>.
 35. Cordat E. Unraveling trafficking of the kidney anion exchanger 1 in polarized MDCK epithelial cells. *Biochem Cell Biol*. 2006;84(6):949–59. <https://doi.org/10.1139/o06-200>.
 36. Xue J, He Q, Xie X, Su A, Cao S. Clinical utility of targeted gene enrichment and sequencing technique in the diagnosis of adult hereditary spherocytosis. *Ann Transl Med*. 2019;7(20):527. <https://doi.org/10.21037/atm.2019.09.163>.
 37. Alloisio N, Texier P, Vallier A, Ribeiro ML, Morlé L, Bozon M, et al. Modulation of clinical expression and band 3 deficiency in hereditary spherocytosis. *Blood*. 1997;90(1):414–20.
 38. Kanzaki A, Hayette S, Morlé L, Inoue F, Matsuyama R, Inoue T, et al. Total absence of protein 4.2 and partial deficiency of band 3 in hereditary spherocytosis. *Br J Haematol*. 1997;99(3):522–30. <https://doi.org/10.1046/j.1365-2141.1997.4263231.x>.
 39. Qin L, Nie Y, Zhang H, Chen L, Zhang D, Lin Y, et al. Identification of new mutations in patients with hereditary spherocytosis by next-generation sequencing. *J Hum Genet*. 2020;65(4):427–34. <https://doi.org/10.1038/s10038-020-0724-z>.
 40. Tole S, Dhir P, Pugi J, Drury LJ, Butchart S, Fantauzzi M, et al. Genotype-phenotype correlation in children with hereditary spherocytosis. *Br J Haematol*. 2020;191(3):486–96. <https://doi.org/10.1111/bjh.16750>.
 41. Aggarwal A, Jamwal M, Sharma P, Sachdeva MUS, Bansal D, Malhotra P, et al. Deciphering molecular heterogeneity of Indian families with hereditary spherocytosis using targeted next-generation sequencing: first South Asian study. *Br J Haematol*. 2020;188(5):784–95. <https://doi.org/10.1111/bjh.16244>.
 42. Tang X, Guo X, Gao J. A novel compound heterozygous mutation in SLC4A1 gene causing severe hereditary spherocytosis and distal renal tubular acidosis. *Indian J Pediatr*. 2020;87(3):233–4. <https://doi.org/10.1007/s12098-019-03171-4>.
 43. Jarolim P, Murray JL, Rubin HL, Taylor WM, Prchal JT, Ballas SK, et al. Characterization of 13 novel band 3 gene defects in hereditary spherocytosis with band 3 deficiency. *Blood*. 1996;88(11):4366–74.
 44. 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(3):379–88. <https://doi.org/10.1042/BJ20091525>.
 45. Shen H, Huang H, Luo K, Yi Y, Shi X. Two different pathogenic gene mutations coexisted in the same hereditary spherocytosis family manifested with heterogeneous phenotypes. *BMC Med Genet*. 2019;20(1):90. <https://doi.org/10.1186/s12881-019-0826-7>.
 46. Ramasamy I. Atypical hereditary spherocytosis phenotype associated with pseudohypokalaemia and a new variant in the band 3 protein. *BMJ Case Rep*. 2020;13(12):e238428. doi: 10.1136/bcr-2020-238428.
 47. Lima PR, Baratti MO, Chiattonne ML, Costa FF, Saad ST. Band 3 Tambaú: a de novo mutation in the AE1 gene associated with hereditary spherocytosis. Implications for anion exchange and insertion into the red blood cell membrane. *Eur J Haematol*. 2005;74(5):396–401. <https://doi.org/10.1111/j.1600-0609.2004.00405.x>.
 48. 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(11):5380–9. <https://doi.org/10.1182/blood-2007-07-099473>.
 49. 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(7):774–83. <https://doi.org/10.1038/ki.2009.258>.
 50. 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(11):1258–63. <https://doi.org/10.1038/ng1656>.
 51. Quilty JA, Reithmeier RA. Trafficking and folding defects in hereditary spherocytosis mutants of the human red cell anion exchanger. *Traffic*. 2000;1(12):987–98. <https://doi.org/10.1034/j.1600-0854.2000.011208.x>.
 52. Maillet P, Alloisio N, Morlé L, Delaunay J. Spectrin mutations in hereditary elliptocytosis and hereditary spherocytosis. *Hum Mutat*. 1996;8(2):97–107.
 53. Iwase S, Ideguchi H, Takao M, Horiguchi-Yamada J, Iwasaki M, Takahara S, et al. Band 3 Tokyo: Thr837→Ala837 substitution in erythrocyte band 3 protein associated with spherocytic hemolysis. *Acta Haematol*. 1998;100(4):200–3. <https://doi.org/10.1159/000040904>.
 54. Sinha R, Agarwal I, Bawazir WM, Bruce LJ. Distal renal tubular acidosis with hereditary spherocytosis. *Indian Pediatr*. 2013;50(7):693–5. <https://doi.org/10.1007/s13312-013-0173-2>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

