Pediatric Hematology Oncology Journal 10 (2025) 100452

Contents lists available at ScienceDirect

Pediatric Hematology Oncology Journal

journal homepage: www.journals.elsevier.com/pediatric-hematology-oncology-journal

A novel *SPTB* variant in a Cambodian child with hereditary spherocytosis without a family history

Chean Sophâl*, Kim Leanghay, Chin Soey

Department of Pediatric Hematology and Immunology | National Pediatric Hospital, Phnom Penh, Cambodia

ARTICLE INFO

Article history:
Received 17 September 2024
Received in revised form
7 April 2025
Accepted 16 April 2025
Available online 19 April 2025

Keywords:
Anemia
Hereditary spherocytosis
Child
RBC membrane disorders
SPTB variant

ABSTRACT

Background: Hereditary spherocytosis (HS) is a form of congenital hemolytic anemia resulting from red cell membrane protein deficiency. Most cases (75 %) will have a family history of HS, but for those with no family history, there may be a delay in diagnosis.
Case report: Herein, we report a 3 ½ years old boy of Cambodian origin who presented with anemia, jaundice, and hepato-splenogaly with no family history of hemolysis. The blood film showed spherocytosis and polychromasia with a negative direct agglutination test (DAT). Genomic DNA analysis of the *SPTB* gene (NM_001355436.2) revealed a novel, unreported heterozygous variant, c.1720dup, (p.Glu574GlyfsTer3), confirming as de novo variant and caused HS. Treatment focuses on supportive care, including folic acid supplementation and transfusion as needed.
Conclusion: This is the first case report of HS resulting from a novel heterozygous *SPTB* variant in Cambodia. HS should be considered in the differential diagnosis of hemolytic anemia, regardless of the patient's ethnic background.

1. Introduction

Hereditary spherocytosis (HS) is the most common form of congenital hemolytic anemia, affecting approximately 1 in every 2000 to 3000 Caucasians, and possibly underdiagnosed in non-Caucasian patients or in milder cases [1,2]. HS is relatively less common in Southeast Asia, with a morbidity rate of 1.27–1.49 per 100,000 [3,4], making it uncommon in Cambodia, and is typically diagnosed between the ages of 2 and 5, though it can present in infancy or delayed until adulthood in milder cases [1,5].

To date, five mutations in genes linked to HS have been identified which encode proteins in the red blood cell cytoskeleton: *SPTA1* (α-spectrin), *SPTB* (β-spectrin), *ANK1* (ankyrin), *SLC4A1* (band 3), and *EPB42* (protein 4.2). Approximately 75 % of HS cases follow an autosomal dominant inheritance pattern, with the remainder arising from autosomal recessive patterns or novel mutations [2,3,5]. We report a case of HS in a Cambodian child with no family history of the condition, who carried a novel heterozygous variant of the *SPTB* gene not previously documented in this population.

2. Case presentation

A 3 ½-year-old boy of Cambodian origin, with non-consanguineous parents, was referred to our center due to intermittent episodes of pallor, jaundice, and dark urine, starting at the age of 1. Since turning 3, he needed blood transfusions every 6–8 weeks. Born to a healthy 29-year-old mother via cesarean section at 41 weeks, he weighed 3720 g at birth and experienced severe neonatal jaundice requiring phototherapy shortly after birth. He is developing normally with an 8-year-old healthy sister. His mother is a carrier of hemoglobin E, while his father has normal hemoglobin levels, with no family history of hemolytic disease.

The physical examination revealed scleral icterus and cutaneous pallor, with no dysmorphic features. The spleen was enlarged 3 cm below the costal margin, and the liver was enlarged 2 cm below the right costal margin. Complete blood count (CBC) showed a hemoglobin level of 5.7 g/dL, a mean corpuscular volume (MCV) of 67 fL, a mean corpuscular hemoglobin concentration (MCHC) of 34 g/dL, a reticulocyte count of 4.6 %, and a platelet count of 159 x 10^9/L. Total and indirect bilirubin levels were 3.3 mg/dL and 2.82 mg/dL, respectively. Both urinary urobilinogen and the Direct Coombs Test were negative, while the G6PD level was normal at 10.2 U/gHb. A peripheral blood smear showed anisocytosis, spherocytes, and

* Corresponding author. National Pediatric Hospital, #100, Federation of Russia Blvd, Phnom Penh, Cambodia.
E-mail address: sophaldes@gmail.com (C. Sophâl).

https://doi.org/10.1016/j.phoj.2025.04.005

polychromasia, with occasional red cell fragments. The other results of blood work were summarily given in Table 1. An abdominal Doppler ultrasound confirmed an enlarged spleen, and enlarged liver without gallstones.

A provisional diagnosis of HS was made based on the clinical and laboratory findings based on peripheral blood smear findings, despite no family history of the disease. We implemented supportive management with packed red cell transfusion as needed, folic acid supplementation, and regular follow-ups.

To clarify the pathophysiology, after receiving his parents' informed consent, genomic DNA analysis was performed at the Medical Genetics Institute in Ho Chi Minh City, Vietnam, targeting hereditary hemolytic anemia genes using a kit from New England Biolabs (USA). DNA fragments from the target gene region were enriched using specific probes IDT DNA (USA) and then sequenced on the NextSeq next-generation sequencing system by Illumina (USA), achieving a high coverage of approximately 100X. At least 95 % of the target gene region had coverage exceeding 10X, with a threshold of 20X applied for germline variant calling. The sequencing results were compared to the GRCh38 reference genome to identify genetic variations. Analysis of the SPTB gene (NM_001355436.2) revealed a heterozygous novel variant, c.1720dup, (p.Glu574GlyfsTer3). The variant found was in the poor signal gene region and was subsequently verified by Sanger testing. The DNA sequences of interest were amplified via PCR on the Applied Biosystems ProFlex™ 3x32-well PCR System, using the synthesized primers. The purified PCR products were then subjected to Sanger sequencing on the 3130xl Genetic Analyzer system, utilizing POP-7™ Polymer (Applied Biosystems). Sanger sequencing confirmed the presence of the heterozygous novel variant c.1720dup (p.Glu574GlyfsTer3). The variant was classified as damaging (FS_CP12) based on analysis with the in-silico prediction tool SIFT Indel. This genetic variant meets several criteria of the ACMG/AMP guidelines, including PVS1, PM2, and PP3, supporting its classification as pathogenic [6]. These results suggest that the SPTB mutation affects the function of the β-spectrin protein, leading to HS (see Fig. 1). Additionally, genomic DNA analysis of the target gene region in both parents returned normal results, confirming that this is a *de novo* variant. Genetic testing also confirmed that the child did not inherit hemoglobin E from the mother.

Table 1
Initial laboratory investigations.

Blood Work	Results	Reference Range
Complete blood count		
White blood cells	8.7	5-15 x 10^9/L
Neutrophils	3.39	1.5-8 x10^9/L
Lymphocyte	4.35	6-9 x 10^9/L
Red blood Cells	2.53	4-5.2 x10^{12}/L
Hemoglobin	5.7 (↓)	11–14 g/dL
Hematocrit	17 (↓)	34–40 %
Reticulocytes	3.6 (↑)	0.2–2 %
MCV	67 (↓)	75-87 fL
MCHC	34 (N)	31–37 mg/dL
Platelet count	159 (↓)	200-490 x 10^9/L
RDW-CV	26 (↑)	11.5–14 %
Peripheral smear	Anisocytosis Spherocytes Polychromasia Red cell fragments	
Liver enzymes		
AST	50	<31 U/L
ALT	20	<32 U/L
Renal functions		
Urea	19	10–50 mg/dL
Creatinine	0.5	0.5–0.9 mg/dL
Biochemistry		
Total bilirubin	3.3 (↑)	0.3–1 mg/dL
Indirect bilirubin	2.82 (↑)	0.2–0.8 mg/dL
Serum iron	12 (↓)	37-148 μ g/dL
Serum ferritin	147	27–375 ng/mL
G6PD level	10.2	5–14 U/g Hb
Hemoglobin electrophoresis		
Hemoglobin A	97.4	95–98 %
Hemoglobin A2	2.6	2.2–3.2 %
Immuno-hematology		
Red Blood Type	A, Rh (+)	
Direct Agglutinin Test	Negative	
Special investigations		
EMA test	Not available	
Osmotic Fragility Test	Not available	

RBCs: red blood cells, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, RDW-VC: red cell distribution width - coefficient of variation, ALT: alanine aminotransferase, AST: aspartate aminotransferase, G6PD: Glucose-6-Phosphate Dehydrogenase, EMA: eosin-5-maleimide binding.

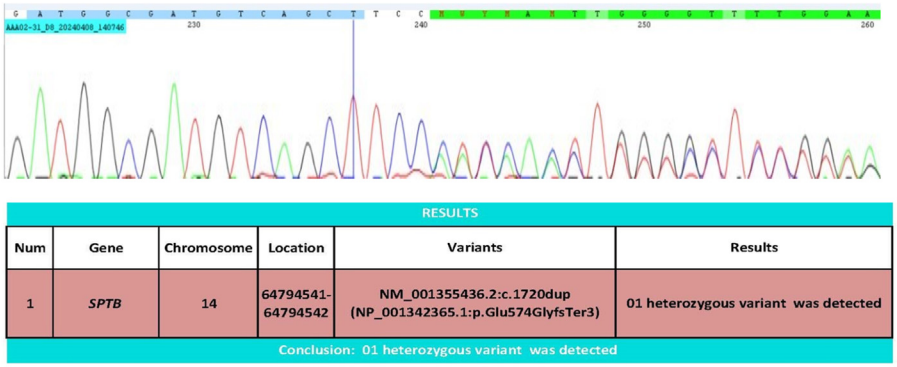
3. Discussion

The diagnosis of HS is generally based on classic clinical features, such as hemolysis, jaundice, splenomegaly, and gallstones, along with the presence of spherocytes, raised mean corpuscular hemoglobin concentration (MCHC), an increase in reticulocytes, a negative direct agglutin test, and a positive family history of the condition [1,7]. Raised MCHC is generally due to the concentration of hemoglobin in spherocytes; however, in the presence of active hemolysis it may appear normal. Mean Corpuscular Volume (MCV) is normal or slightly decreased due to compensatory changes in erythropoiesis in the bone marrow, or coexisting early iron deficiency anemia as seen in our case [1,5]. The clinical manifestations of HS are similar to those seen in patients with other forms of anemia, such as thalassemia, autoimmune hemolytic anemia, and red cell enzyme defects (e.g., G6PD deficiency) and excluding rare causes of congenital hemolytic anemia can be challenging [8]. Patients with different HS types may exhibit varied severity, and our patient would be considered severe HS according to the following criteria [1,8,9]:

- Mild HS: hemoglobin 11–15 g/dL; reticulocyte count 3–6 %; bilirubin 1–2 mg/dL; few spherocytes.
- Moderate HS: hemoglobin 8–12 g/dl; reticulocyte count >6 %; bilirubin >2 mg/dL; 5 %–20 % spherocytes.
- Severe HS: hemoglobin 6–8 g/dL; reticulocyte count >10 %; bilirubin >2 mg/dL; 20 %–30 % spherocytes.

The diagnosis of HS can be confirmed by the EMA (eosin-5-maleimide binding) test, and the osmotic fragility test, neither of which were performed in this case [7,8]. Some patients with HS may present with hematologic crises, which may be hemolytic, aplastic, or megaloblastic. The aplastic crises are associated with parvovirus B19 infection. In these cases, hemoglobin and reticulocytes will decrease first, followed by a reduction in erythroblasts in the bone marrow [7]. The megaloblastic crises, on the other hand, are caused by folate deficiency and can be corrected by adequate folate supplementation [7].

In our clinical experience in Cambodia, infants and toddlers exhibiting marked jaundice, anemia, and splenomegaly are frequently presumed to have thalassemia or chronic malaria., particularly in the absence of a family history suggestive of hereditary spherocytosis, as was observed in the present case. Our case reminds us it is important to consider RBC membrane defects, such as HS and Southeast Asian ovalocytosis (SAO), which are known to exist in the region. A peripheral blood smear can help differentiate between these two disorders: SAO is characterized by oval-shaped red blood cells and stomatocytes while HS features spherocytes , as seen in our patient. In approximately 75 % of cases, there is a



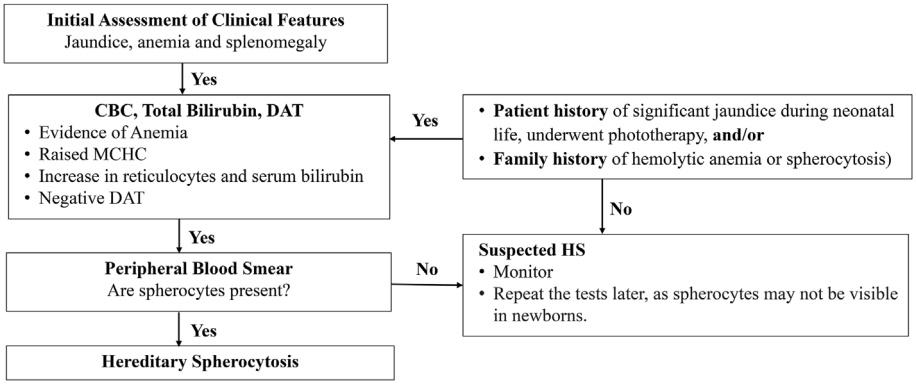
RESULTS

Num	Gene	Chromosome	Location	Variants	Results
1	*SPTB*	14	64794541-64794542	NM_001355436.2:c.1720dup (NP_001342365.1:p.Glu574GlyfsTer3)	01 heterozygous variant was detected

Conclusion: 01 heterozygous variant was detected

Image courtesy of Medical Genetics Institute at Ho Chi Minh City of Vietnam

Fig. 1. Patient's genomic DNA analysis by Sanger Sequencing.



Flowchart 1. A feasible and low-cost evaluation of HS.

family history of HS [1]; however, in cases without a family history, misdiagnosis can occur and our case initially appeared to resemble thalassemia, which is common in Cambodia – particularly since the patient's mother was heterozygous for hemoglobin E. Interestingly, Ang and Lam reported that only half of Singaporean patients with HS had a positive family history in their cohort [3]. Children with a family history of HS who exhibit typical clinical features—such as jaundice, anemia, and splenomegaly—along with laboratory findings (e.g., spherocytes, elevated MCHC, and an increased reticulocyte count) do not require any additional testing (grade A evidence) [1]. In most cases, HS can be diagnosed through a simple and cost-effective evaluation, especially in settings where the EMA test is unavailable, such as Cambodia (**see** Flowchart 1).

The specific genetic mutation influences the severity of symptoms, the age at which they appear, and the response to treatments such as splenectomy. However, genetic testing is not routinely offered; it is usually performed only for patients who exhibit an unusually severe phenotype, such as those requiring frequent blood transfusions at a young age [3]. The genotype-phenotype correlation in HS is complex and involves specific mutations in key erythrocyte membrane proteins. [10]. Among these genetic mutations, ANK1 mutations are the most common, accounting for about 50 % of cases, followed by SPTB mutations, which represent approximately 30 % [7,11]. This pattern of variant distribution aligns with what is seen in other Asian countries but differs from that observed in non-Asian countries [7]. The exact prevalence of HS in Cambodia is currently unknown; while cases are likely encountered in clinical practice, none have been officially documented or published.

Treatment primarily focuses on supportive care, including folic acid supplementation and blood transfusions. For children who are moderately to severely affected, a splenectomy can effectively reduce hemolysis and increase red blood cell lifespan, ideally in fully immunized children over the age of 5 [1]. If symptomatic gallstones are present, the gallbladder should be removed alongside the spleen during surgery [1]. It is important to note that splenectomy increases the lifelong risk of overwhelming infection, especially from pneumococcal bacteria. This risk is only partially reduced by receiving pre-operative vaccinations and following post-splenectomy antibiotic prophylaxis [1].

4. Conclusion

This report presents the first case of HS caused by a novel heterozygous variant in the *SPTB* gene in Cambodia. We aim to raise awareness among physicians about the rare possibility of HS in our community to promote early diagnosis and effective management, enhancing outcomes for pediatric patients. HS should be considered in the differential diagnosis for any patient with symptoms of hemolytic anemia, regardless of ethnic background.

Patient's consent

Informed consent was obtained by the child's parents and they agreed to the case publication.

Consent for publication

Informed consent was obtained by the child's parents and they agreed to the case publication.

Ethical statement

University of Health Sciences, Phnom Penh, Cambodia granted approval to this study (no. 2625 UHS, dated on September 23, 2022).

Funding statement

Not applicable.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgment

The authors would like to thank Dr. Meredith Wiggins, B. Tech, MBBS, FRACP, FRCPA, for editing a draft of this manuscript, and Dr. Nguyen Thanh Ngoc Binh for her contributions to genetic interpretation. We also express our gratitude to the patient and their parents for kindly providing consent for the case presented in this paper.

References

[1] Bolton-Maggs PH, Langer JC, Iolascon A, et al. General haematology task force of the British committee for standards in H. Guidelines for the diagnosis and management of hereditary spherocytosis-2011 update. Br J Haematol 2012;156:37–49.
[2] Da Costa L, Galimand J, Fenneteau O, Mohandas N. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev 2013;27(4):167–78.
[3] Ang SH, Lam JCM. Hereditary spherocytosis in an Asian children's hospital. HK J Paediatr (new series) 2022;27:113–7.
[4] Wang C, Chi 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;4:76–81.
[5] Sanli Celik S, Genc DB, Yildiz Yildirmak Z. Clinical characteristics and treatment outcome of hereditary spherocytosis: a single center's experience. Med Bull Sisli Etfal Hosp 2023;57(4):531–5.
[6] Tavtigian SV, Harrison SM, Boucher KM, Biesecker LG. Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines. Hum Mutat 2020;41(10):1734–7.
[7] Shih Y-H, Huang Y-C, Lin C-Y, et al. A large family of hereditary spherocytosis and a rare case of hereditary elliptocytosis with a novel SPTA1 mutation underdiagnosed in Taiwan: a case report and literature review. Medicine 2023;102:4 (e32708).
[8] Wu Y, Liao L, Lin F. The diagnostic protocol for hereditary spherocytosis-2021 update. J Clin Lab Anal 2021;35:e24034.
[9] Sabharwal KA, Simon MW. Hereditary spherocytosis: review of cases and discussion of hematologic characteristics and updated diagnostic testing. J Adv Pediatr Child Health 2023;6:7–13.
[10] He BJ, Liao L, Deng ZF, Tao YF, Xu YC. Lin F.Q.Molecular genetic mechanisms of hereditary spherocytosis: current perspectives. Acta Haematol 2018;139(1):60–6.
[11] Yamamoto KS, Utshigisawa T, Ogura H, Aoki T, Kawakami T. Ohga S. et al. Clinical and genetic diagnosis of thirteen Japanese patients with hereditary spherocytosis. Hum Genome Var 2022;9:1.