A novel *SPTB* gene mutation in neonatal hereditary spherocytosis: A case report

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Abstract. The aim of the present study was to enhance the understanding of the diagnosis and treatment of neonatal hereditary spherocytosis (HS). Gene sequencing and analysis was performed for the crucial splicing signals on the exons and introns of the 302 known pathogenic genes [including *ANK1, SPTAN1, SPTA1, EPB42, SLC4A1*, and *SPTB*] that are associated with this genetic deficiency of erythrocytes. A 26-day-old female presented with jaundice, anemia, an increased count in peripheral blood reticulocyte and spherocytes and a positive acidified glycerol hemolysis test. Gene sequencing revealed a novel mutation of c.3737delA (p.Lys1246fs) in the exon 16 of *SPTB* (14q23|NM_000347.5) gene in the patient and her father. The mutation was a frame-shifting mutation, which may result in the truncation of β-haemoglobin in the erythrocyte membrane can lead to loss of normal function, leading to the occurrence of diseases, including jaundice and hemolytic anemia. For neonates with jaundice and anemia, family history, erythrocyte index and peripheral blood smear findings have been indicated to contribute to the diagnosis of HS. In the current study, gene sequencing was indicated to be helpful for the diagnosis of HS. A novel mutation of *SPTB* gene was identified, which may be pathogenic via modulating the activity of β-spectrin in the erythrocyte membrane.

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Introduction

Hereditary spherocytosis (HS), which is also known as Minkowski-Chauffard disease, is a heterogeneous disease and a type of non-immune hemolytic anemia that is identified by spherocytes in the peripheral blood smear of patients. The clinical manifestations of HS include anemia, jaundice and splenomegaly. Most of the neonates with HS present with jaundice at the early stages of the disease, which then progresses into severe anemia (1). For the neonates with HS aged <1 year, the clinical manifestations are usually severe, with the majority of patients showing initial jaundice and subsequent severe anemia. In contrast, in the affected neonates aged >1 year, the conditions showed gradual attenuation (2). According to previous studies (3-7), early-stage diagnosis and treatment contribute to the reduction of adverse events. In North Europe and North America, the prevalence of HS in the neonates was 1/5,000 and 1/2,000, respectively (4). In mainland China, the prevalence of HS in male neonates (<1 year old) was indicated to be 0.18/1 million, while the prevalence was 0.19/1 million among female counterparts between January 1987 and December 2013 (2). To date, in hospitals on a country level or even from communities, which accounts for ~70% of the country's medical resources, only ~3% of total number of HS cases are diagnosed (2). The present study reports on a neonate with HS, and summarizes the clinical manifestations, laboratory test findings and gene sequencing data gained. This report will contribute to the understanding and diagnosis of the HS in neonates and expand the spectrum of *SPTB* gene mutations.

Case report

A 26-day-old female neonate was admitted to Tianjin Children's Hospital due to jaundice on February 13, 2018. The neonate was born via spontaneous delivery at a gestational age of 41 weeks. After birth, the neonate was breast-fed and the defecation of the neonatal meconium was normal. Jaundice was noticed ~24 h post-delivery and phototherapy lasting for 2 days was subsequently performed at Nanpi County Hospital, Hebei Province, China. The condition showed slight attenuation. No obvious remission or deterioration was noticed when the patient was admitted to the hospital. No urine that was dark

brown in color, kaolin stools or torsion spasm were observed. The patient's father had a history of anemia, and received cholecystectomy due to gallstones. The patient's mother had no history of haematological system diseases.

On physical examination, the body temperature of the patient was 36.5˚C, the respiration rate was 50 breaths/min, the pulse was 150 beats/min and the blood pressure was 65/35 mmHg. The blood oxygen saturation level was 94%. According to the standard of growth curves for Chinese children and adolescents which contains weight, length/height, head circumference, weight-for-length/height and body mass index aged 0-18 years, the child's nutrition and development were normal (8). The results of neonatal behavioral neurological assessment showed that the child's mental response was satisfactory. Ochrodermia was observed across the whole body. Estimation of bilirubin according to the location of jaundice on the skin, the bilirubin value of the child was close to 5-10 mg/dl. No edema was observed. The doctors in the department of neonatology carried out physical examination on the patient and no positive signs were indicated, such as lassitude, feeding difficulty and hepatospleno-megaly. For the blood routine examination, the hemoglobin level was 51 g/l (normal range, 110-160 g/l), the mean corpuscular volume (MCV) was 81.5 fl (normal range, 86-100 fl), the mean hemoglobin was 29.3 pg (normal range, 26-31 pg), the mean hemoglobin concentration was 384 g/l (normal range, 210-370 fl) and the red cell distribution width was 53.8 fl (37-50 fl). The results of cell counting demonstrated that a total of 10 interme-diate erythroblasts and 3 acidophilic erythroblasts were identified per 100 leukocytes. These were counted manually using an Olympus CX21 optical microscope (100X oil immersion objec-tive; Olympus Optical Co, Ltd.). The proportion of reticulocyte was 12.3%. The number of leukocyte was 11.03×10^9/l. The proportion of lymphocytes, neutrophils, monocytes, eosinophils and basophils was 60, 31, 7, 1 and 1%, respectively. The platelet count was 333×10^{11}/l. The concentration of C-reactive protein was <2.5 mg/l. The results of the blood smear test indicated that the morphology of the erythrocytes was different, the spherocyte was smaller than the normal erythrocyte (Fig. 1), in which sphero-cytes accounted for ~5% in total. Hemolysis findings revealed that the proportion of alkali-resistant hemoglobin was 47.97%, while the acidified glycerin hemolysis test (AGLT) was 110 sec (control, 290 sec). No aberrant changes were identified in the hemo-globin electrophoresis and glucose-6-phosphate dehydrogenase (G-6-PD) activity. The concentration of ferritin was 588.6 μg/l. The folic acid, vitamin B12 and serum iron were within normal ranges (folic acid 5-6 μg/l, vitamin B12 200-800 ng/l, serum iron 12.8-31.3 μmol/l). The unsaturated iron and the total iron-binding capacity were 23 μmol/l (normal range, 26-51 μmol/l,) and 50 μmol/l (normal range, 55-77 μmol/l), respectively, which were lower than the normal ranges. For the biochemical analysis, the direct bilirubin, indirect bilirubin, and lactate dehydrogenase was at a concentration of 11.2, 139.8 and 553 U/l, respectively. The liver and renal function was normal, and the results of blood gas analysis and electrolyte findings were normal. A serological test for hemolysis indicated that the patient was of type O blood group (RHD positivity). The patient's mother was blood type A (RHD positivity). The direct antiglobulin test, free antibody test and antibody release test were all negative. No abnormalities were noticed in the TORCH test. The examinations of auto-antibodies, electrocardiogram, echocardiography and imaging examinations

of the chest and abdomen were within their normal ranges. Ultrasonography indicated no abnormalities in the liver, gall-bladder, spleen, kidneys or brain.

Upon admission, the patient received phototherapy. Type O washed red blood cells were supplemented to correct the anemia, together with supporting therapy. The whole treat-ment duration was four days. The jaundice showed remission, and the anemia was corrected. Finally, the patient exhibited a satisfactory outcome. In the 9-month follow-up, the patient received erythrocytes supplementation due to anemia at months 3 and 7, respectively. No jaundice, splenectasis or liver dysfunction were observed.

Genetic analysis. The genomic DNA was extracted from the patient's and her parents' peripheral blood using the QIAamp blood DNA mini kit (QiagenGmbH) following the manufacturer's protocol. The exome sequencing kit (xGen® Exome Research Panel; Integrated DNA Technologies, Inc.) was used for the preparation of the sequencing library. The generated library was analyzed on the NextSeq 500 analyzer (Illumina, Inc.) for the sequencing of the exons of the 302 genes associated with hematological system disease related genes (such as *ANK1*, *SPTA1*, *EPB42*, *SLC4A1* and *SPTB*). The mean sequencing depth was 100X. Sequencing data was processed using Burrows-Wheeler-Aligner forhg19 reference sequence (9) alignment and a Genomic-Analysis-Toolkit (V4.0.6.0) for variant calling. Variants annotation was performed using Annovar (V20180118) (10).

According to the high-throughput sequencing results, the *SPTB* mutation was verified using Sanger sequencing based on the neonatal and parental DNA samples. Specific primers were designed using the Primer Premier 5.0 software (PREMIER Biosoft) for the coding region of the exon of the target gene (forward, 5'-CCGCTCATGGAATCCCAC-3'; reverse, 5'-GGAGTAGTGCCTCCTCCCTG-3'). PCR amplification was performed using the 2XPCR Master mix polymerase (Qiagen, GmbH) on an ABI 9700 PCR facility (Thermo Fisher Scientific, Inc.). The thermo cycling conditions of PCR were as follows: 95˚C for 10 min, followed by 35 cycles of 95˚C for 30 sec, 60˚C for 30 sec and 72˚C for 1 min, and final exten-sion at 72˚C for 7 min. Following DNA purification, Sanger sequencing was performed on the ABI3500 analyzer (Thermo Fisher Scientific, Inc.), followed by sequencing comparison with the reference sequences.

Based on the determination of the crucial splicing signals on the exons and introns of the 302 known pathogenic genes (such as *ANK1*, *SPTA1*, *EPB42*, *SLC4A1* and *SPTB*) for the genetic deficiency of erythrocyte, a heterozygous pathogenic gene was identified in the *SPTB* gene. A mutation of the *SPTB* gene, c.3737delA (p.Lys1246fs), was identified in exon 16. Additionally, the same mutation was identified in the patient's father (Fig. 2). The mutation was a type of frame-shift muta-tion, resulting in frame-shifting from Lys1246. The mutation may lead to loss of normal function due to termination of the coded protein. Moreover, this mutation was not available in the HGMD database (http://www.hgmd.cf.ac.uk/ac/index. php), ESP6500 (https://evs.gs.washington.edu/EVS/), 1000 G (http://www.1000genomes.org/) or dbSNP (https://www.ncbi. nlm.nih.gov/snp/?term=) database. Overall, c.3737delA on the exon 16 of *SPTB* identified in the present study was a novel
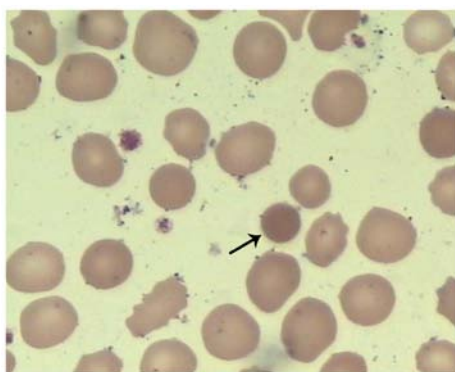
Figure 1. Morphology of erythrocytes in the blood smear (Wright-Giemsa stain; magnification, x1000). The spherocyte is indicated by the black arrow.
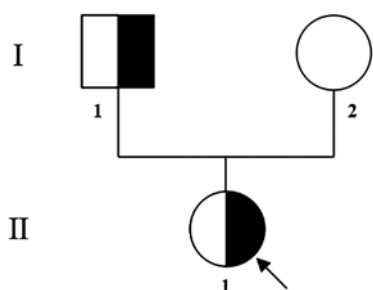

Figure 2. Family genetic pedigree. The mutation c.3737delA of *SPTB* gene was identified in the proband (indicated by arrow) and her father. Sanger sequencing of the mother showed wild-type.
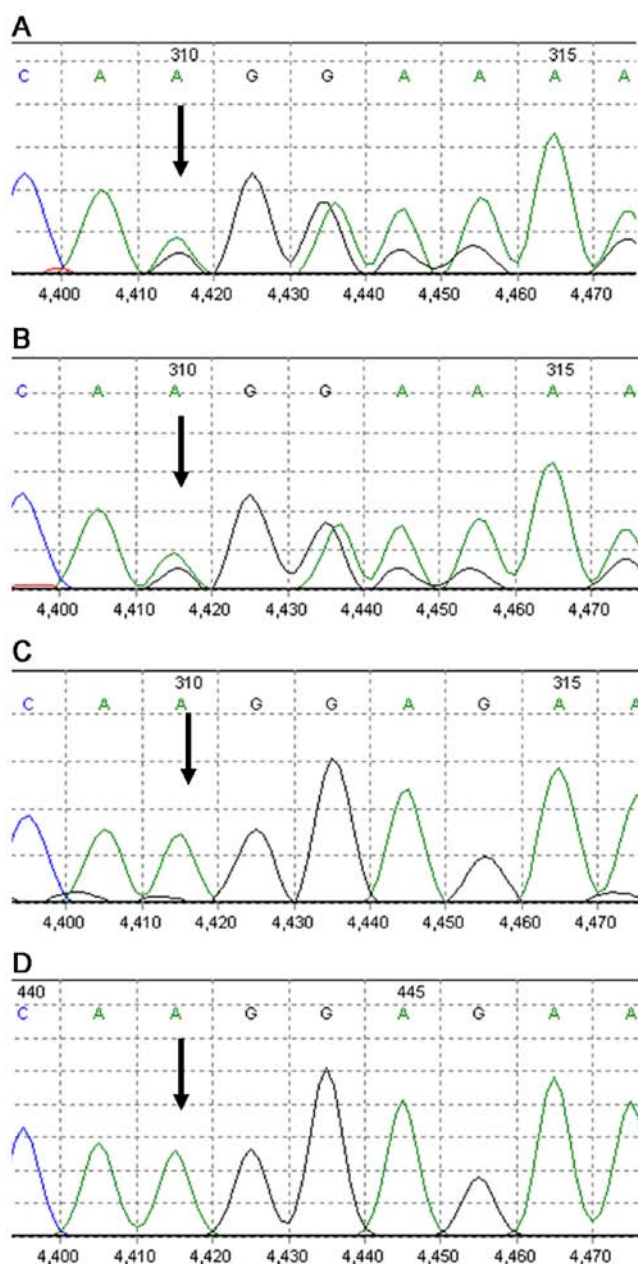

Figure 3. Gene sequencing results of the mutation c.3737delA of *SPTB* gene in the (A) child, (B) her father, (C) her mother and the (D) referencing sequences.

pathogenic mutation. Sequencing data of the *SPTB* gene in the patient and her father are presented in Fig. 3. The patient was diagnosed with type 2 HS, which was divided into autosomal dominant (AD) inheritance.

Prediction of protein structure. The SWISS-MODEL homology modeling server (https://www.swissmodel.expasy.org/) was used to predict and compare the spatial structure of the wild-type protein and the protein encoded by the mutated genes. Meanwhile, the effects of the *SPTB* gene mutation on coding protein were justified, in order to speculate the pathogenicity of the new protein.

Fig. 4A indicates the results of the prediction of the tertiary structure of the SPTB protein (817-1265) using the SWISS-MODEL software. The predicted template was Protein Data Bank ID number 4uxv.1.A. Fig. 4B summarizes the prediction data of the protein sequence with a mutation on residue 1246. Compared with the tertiary structure of wild-type protein, the c.3737delA (p.Lys1246fs) mutation resulted in premature termination of the protein, triggering the loss of the subsequent α-spiral.

Discussion

HS, which affects many individuals worldwide, exhibits a prevalence of 27.6 per million within the Chinese population (2). In neonates aged <1 year, the prevalence of HS is ~0.37/1 million with a ratio of 1:1 in male and females (2). However, a higher trend of misdiagnosis is reported among these patients, and

some patients with moderate manifestations are not diagnosed in clinical settings (6). Due to this, the number of patients may be higher than expected (11).

The pathogenesis of HS may be associated with the deficiency of a variety of membrane proteins of the erythrocytes, including ankyrin-1, band 3, *SPTB*, α-spectrin and protein 4.2, which result in the decline of the surface area of the erythrocyte membrane in patients with HS (12). Meanwhile, the damage of erythrocytes with poor deformation capacity in the spleen of individuals with HS is a major cause of hemolysis (8). According to the genetic deficiency of erythrocyte membrane proteins, HS is divided into five types (Table I), among which type 3 and 5 are of autosomal recessive (AR) inheritance with a lower prevalence (3,13). In this case, the patient was diagnosed with type 2 HS, which was divided into AD inheritance.
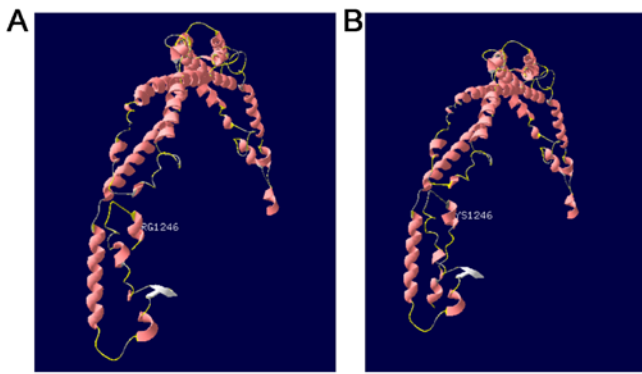
Figure 4. *SPTB* protein structure prediction using the SWISS-MODEL software. (A) normal protein tertiary structure. (B) Tertiary structure of mutated protein.

The typical features of HS include anemia, jaundice, splenomegaly and ceticulocytosis (2). The severity of HS is divided into asymptomatic state, mild, moderate and severe, according to the degree of anemia (14). The majority of patients exhibit mild HS, and up to 20-30% present with a purely compensated hemolysis due to the balance between reticulocyte production and red cell destruction (15). Approximately 50% of neonates with HS are anemia-free at post-natal week one, and rare cases exhibit splenomegaly (16). Jaundice is the most common manifestation for neonatal HS (3,15,17). Neonatal jaundice usually occurs within a few post-natal days. The hemoglobin concentration would be in the normal range, cases may develop transient or even severe anemia within a few post-natal weeks, due to inadequate compensation of the splenic filtration function caused by the lack of appropriate reticulocytes (7). Most of these conditions exhibit remission within 12 months post-partum (14). In the present study, the neonate with HS exhibited delayed remission of jaundice and severe anemia without kernicterus. The patient was followed up for 9 months, and blood transfusion was required to correct the anemia.

Clinical manifestations, family history and peripheral blood smear findings are relied upon in the diagnosis of HS. For the blood smear, patients with HS exhibit alternations of spherocyte proportion that are associated with the severity of anemia, as well as presence of mushroom-shaped erythrocytes, poikilocytosis and acanthocyte (18). According to the HS diagnosis guidelines that are proposed by the British Committee for Standards in Haematology (6), additional tests are not recommended for patients with HS and with typical clinical manifestations and laboratory findings. As the clinical manifestations in the neonatal patients with HS are not typical, and some patients usually present spherocytes, the diagnosis of HS in these patients is still difficult (14). A parental history of HS has been reported in the majority (65%) of the neonates with HS (16). Therefore, determining the parental history of anemia and/or the family history of anemia, jaundice, splenectomy or early-stage cholelithiasis in these neonates with jaundice is crucial for the diagnosis of HS in clinical practice (3). In addition, the eosin-5-maleimide binding test, osmotic fragility test, osmotic gradient ektacytometry, AGLT and pink test all contributed to the diagnosis of HS in clinical practice (11,19,20). For some patients, a genetic test is required to assist the diagnosis (14,17). In the present study, the patient's father exhibited anemia with a family history of cholelithiasis, which was clinically manifested as delayed remission of jaundice and severe anemia. Finally, diagnosis of HS was confirmed based on laboratory findings and the results of the genetic analysis.

HS is a rare disease of genetic deficiency that lacks appropriate treatment options. Currently, its treatment is mainly focused on the control of its severity (2). Phototherapy, which lowers the bilirubin in the neonatal HS, is considered to be the major treatment therapy in the early post-partum period (16). Moreover, treatment should begin immediately to those with a high level of bilirubin or a higher medium than the risk zone (>75th percentile zone). Furthermore, according to the guidelines proposed by the American Academy of Pediatrics (3), further blood exchange transfusion is required. In cases of signs of anemia, blood transfusion may also be required. Since erythropoiesis is damaging at a certain post-partum period (1-4 weeks) (16), single administration of erythropoietin could be used, or utilized simultaneously with blood transfusion. Folic acid supplementation should be considered for those with moderate and severe HS in order to prevent the complications associated with folic acid deficiency (16). Splenectomy is not recommended for ~12 months after delivery (16). Splenectomy is effective for treating moderate and severe HS, however, it may lead to trauma, decline of immunity, and pulmonary hypertension that is induced by arterial and venous thrombosis (5,14). Total splenectomy may therefore be more effective than partial splenectomy (2,21-24). Individual follow-up schemes should be established for children with HS, which are based on the severity of anemia and the monitoring of the growth and development (16). Meanwhile, care should be taken regarding iron overload in the children who undergo persistent blood transfusion (3).

The *SPTB* gene, which encodes for the β subunit of spectrin, is a member of the spectrin gene family. It is localized on 14q23.3 with a length of 100 kb, consisting of 35 exons, and its encoded proteins form the cytoskeletal superstructure of the erythrocyte plasma membrane (25). Upon binding with ankyrin, spectrin serves a crucial role in the formation and stability of the erythrocyte membrane. The *SPTB* gene mutation is associated with type II spherocytosis, hereditary elliptocytosis and hemolytic anemia of neonates (26,27). In general, the AD pattern was reported in 75% of patients with HS, while in the remaining 25%, the AR pattern was exhibited, or the disease was due to denovo mutations (5). Moreover, the inheritance patterns of some *SPTB* mutations are unknown (28). In the present study, the patient's father carried the similar mutation of the *SPTB* gene, which was not identified in the patient's mother. The patient's father had a history of anemia and cholelithiasis. Therefore, it was proposed that this novel mutation in *SPTB* gene was of AD inheritance, Therefore, the risk of HS was speculated to be up to 50% in the second child of this family.

In general, the common mutation types of *SPTB* gene include nonsense mutations, frame-shifting mutations and splice site mutations, which give rise to mRNA defects and truncated β-spectrin (29). In this case, a novel mutation of c.3737delA (p.Lys1246fs) in the *SPTB* gene was identified as a frameshift mutation, which led to premature termination of

Table I. Correlation between the gene and phenotype of the HS.

Type	Gene	Gene location	Protein	Genetic type	Percentage (%)	Severity
Type 1, OMIM:182900	ANK1 (612641)	8p11.21	Ankyrin-1	Autosomal dominant	40-50	Mild and moderate
Type 2, OMIM: 616649	SPTB (182870)	14q23.3	β-spectrin	Autosomal dominant	15-30	Mild and moderate
Type 3, OMIM: 270970	SPTA1 (182860)	1q23.1	α-spectrin	Autosomal recessive	<5	Severe
Type 4, OMIM: 612653	SCL4A1(109270)	17q21.31	Band-3	Autosomal dominant	20-35	Mild and moderate
Type 5, OMIM: 612690	EPB42 (177070)	15q15.2	Protein 4.2	Autosomal recessive	<5	Mild and moderate

HS, hereditary spherocytosis.

Table II. Frequency of mutation types in SPTB gene in different populations.

Mutation types (%)	Missense	Nonsense	Frame shift	Splicing mutation	Other types	No. of mutations	(Refs.)
Netherlands	21.4	42.9	14.3	-	21.4	14	(28)
Korea	9.1	36.4	54.5	-	-	11	(33)
Chinese Mainland	11.8	47.1	23.5	17.6	-	17	(34)
Chinese Mainland	33.3	33.3	-	33.3	-	3	(35)
Chinese Mainland	18.75	50.0	18.75	12.5	-	16	(36)

-, No frame shift mutation was identified.

the protein and the loss of the subsequent α-spiral. Similarly, the identified mutation, expected to encode for a truncated protein, was usually the pathogenic one (5,30,31). In addition, the mutation types in other populations was reviewed (Table II). It was concluded that the most common mutation types are nonsense and frame-shifting mutations. This provides a direction for further exploration of the instability and degradation of mRNA with premature termination codons.

The homology modeling technique is a mature technique that is commonly utilized in structural biology, and can notably reduce the differences between the predicted and actual protein sequences (32). The SWISS-MODEL software is the first homology modeling server of protein that is completely automatized to date (30). In the present study, The SWISS-MODEL software was utilized for the analysis of the spatial structure of the protein sequence encoded by SPTB gene. Compared with the predicted tertiary structure of the wild type protein, the α-spiral was no longer available, due to premature termination of protein transcription induced by the c.3737delA (p.Lys1246fs) mutation. Such spatial alteration of the protein may finally result in functional changes of β-spectrin in the erythrocyte membrane, which then can induce the onset of the disease.

In the present study, a novel mutation of SPTB is reported. The SWISS-MODEL software was used to analyze the spatial structure of the protein encoded by the SPTB gene, without validating it using laboratory data, which will be focused on in future studies. During the follow-up, genetic communication and suggestions were given to the parents of the patients, together with informing the guidelines about giving birth to a new child, in order to reduce the prevalence of HS. In summary, the clinical manifestations, laboratory findings and gene sequencing results of one neonatal HS case was reported in the current study. Furthermore, the epidemiological features, clinical manifestations, diagnosis and treatment of HS were summarized. In cases of neonates with severe hyperbilirubinemia, special attention should be paid to the family history, erythrocyte index and findings of the peripheral blood smear test. For the icterohemolytic neonates, the bilirubin should be monitored strictly, together with appropriate treatment. Particularly, the hemoglobin must be monitored in the post-partum period between1 week to 1 month. Gene sequencing also contributes to the diagnosis of this disease. The identified c.3737delA (p.Lys1246fs) mutation results in loss of α-spiral, after prediction of the tertiary structure of protein. This may lead to the dysfunction of β-spectrin in the erythrocyte membrane, triggering corresponding changes in the clinical and laboratory test findings. Early diagnosis and treatment may decrease the severity and poor outcome among patients with HS.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and JZ collected and analyzed the data, and wrote the manuscript. LS, CS and NL collected the clinical data. JS and YF predicted the protein structure. GL and JS participated in making substantial contributions to the conception and design, drafting and revising the important intellectual content of manuscript All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocols were approved by the Ethical Committee of Tianjin Medical University General Hospital.

Patient consent for publication

Consent for publication was obtained from the patient's family.

Competing interests

The authors declare that they have no competing interests.

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