Novel SPTB frameshift mutation in a Chinese neonatal case of hereditary spherocytosis type 2: A case report

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Abstract. Hereditary spherocytosis (HS) is an erythrocyte membrane disease with a non-specific phenotype, particularly occurring in neonatal patients, and its diagnosis is challenging. The present study reports on a patient with neonatal HS and reviewed the genetic characteristics of reported neonatal HS cases in China. The patient was admitted only a few hours after birth with jaundice. Auxiliary examination indicated anemia and hyperbilirubinemia. Spherical erythrocytes were occasionally observed in peripheral blood smears. Genetic testing suggested that the patient harbored a novel frameshift mutation (p.Asp495fsTer78) in spectrum, β, erythrocytic (SPTB), which was carried by the father. Review of 160 cases of HS in China revealed 24 to be neonatal cases. In these neonatal cases, the frequency of ankyrin 1 (ANK1) mutations and loss-of-function mutations of pathogenic genes (including ANK1 and SPTB) was higher than that in the non-neonatal group. In conclusion, the present study further expanded the mutation spectrum of SPTB and reaffirms the diagnostic value of gene detection in neonatal HS.

Introduction

Hereditary spherocytosis (HS), a type of erythrocytosis, is heterogeneous in terms of clinical manifestations, biochemical data and genetics (1). It is a common hereditary hemolytic anemia in Nordic populations and has been reported in other backgrounds, but the global incidence rate is unknown (2). The main clinical features of HS are anemia, jaundice, reticulocytosis, splenomegaly and spherical erythrocytes, which may be observed in peripheral blood smears (3). Patients with severe HS may be of short stature and have a delayed puberty (4). The phenotypic severity of HS is related to different pathogenic gene defects and may start at any age, particularly in the neonatal stage. A phenotype characterized by hyperbilirubinemia may lead to severe jaundice. In addition, according to guidelines for the diagnosis and management of HS (2011 update), HS may be suspected when the mean corpuscular hemoglobin concentration (MCHC) in newborns exceeds 360 g/l (5). However, according to the reported Asian cases, an increase in MCHC is not common in neonatal HS (3,6-8). This may be due to differences in ethnic background or statistical errors caused by lower numbers of neonatal cases. The present study reported a case of neonatal HS in a Han Chinese pedigree. The patient developed jaundice and hyperbilirubinemia after birth, but MCHC remained within the reference range. Gene detection revealed a novel frameshift mutation in spectrum, β, erythrocytic (SPTB). In conclusion, the present case report further expands the variation spectrum of SPTB and provides reliable clinical data for the study of the mechanisms underlying HS.

Case report

Case presentation. The patient was a male neonate and the first child of the mother. At the gestational age of 40^{+3} weeks, the infant was born through natural vaginal birth. The birth weight was 3,420 g. The Apgar score was 10 points at both 1 and 5 mins post-birth. In February 2022, 10 h after birth, the infant was admitted to the Department of Neonatology of the Fourth Affiliated Hospital of Anhui Medical University (Hefei, China) due to 'abnormal skin yellowing'. Physical examination revealed lethargy, yellow colour of the skin and mucous membranes of the whole body and no splenomegaly. Routine blood examination indicated that symptoms of anemia and jaundice progressively aggravated, mainly due to the continuous decrease in red blood cell count (RBC) and hemoglobin. Biochemical examination revealed an abnormal increase in the reticulocyte count and reticulocyte ratio, as well as aspartate aminotransferase, alanine aminotransferase and total bilirubin levels (Table I). Peripheral blood smear revealed that the size of mature RBCs varied and that spherical RBCs were occasionally observed. Results of the anti-globulin (Coombs') test and glucose-6-phosphate dehydrogenase screening were negative. No abnormality was found on B-ultrasonography of the head or abdomen. The patient was hospitalized for 17 days without being treated with blood transfusion. After phototherapy (12 h

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per day for 4 days), the skin yellowing gradually improved. The main indication for discharge was the stable level of total bilirubin <15 μg/l; furthermore, symptoms associated with anemia, such as shortness of breath and laborious feeding, were not observed. The parents of the patient were unrelated (the father was 30 years old and the mother was 29 years old when the patient was hospitalized). The father had a history of anemia and splenomegaly, and after splenectomy (at 15 years of age), his anemia symptoms resolved.

Genetic analysis. To further clarify the pathogenic factors of the patient's clinical phenotype, ~3 ml of peripheral blood was extracted from the patient and the patient's parents for trio-whole-exome sequencing testing. The work described in this case report was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The ethics committee of the Fourth Affiliated Hospital of Anhui Medical University (Hefei, China) approved the study and the publication of this report (PJ-YX2022-008). The parents of the patient provided written informed consent regarding the publication of the medical data and images of the case. In brief, the leukocyte genome was extracted according to the instructions of a DNA extraction kit (CoWin Biosciences). After constructing the exome library, the Illumina Novaseq 6000 series sequencer (Illumina, Inc.) was used for high-throughput sequencing. The sequencing data were subjected to quality control, reference sequence comparison and screening, normal population distribution frequency analysis (dbSNP, www.ncbi.nlm.nih.gov/snp/; ExAC, www.exac.broadinstitute.org/; and 1000 Genomes, www.1000genomes.org/), and pathogenicity prediction analysis using various software (SIFT, www.sift.bii.a-star.edu.sg/; Polyphen-2, www.genetics.bwh.harvard.edu/pph2/; and MutationTaster, www.mutationtaster.org/).

The results indicated that the patient harbored a frameshift mutation (NM_001024858: c.1484dela/p.asp495fster78); the patient's father carried a heterozygous mutation and the patient's mother carried the wild-type gene. There were no pathogenic mutations in other hemoglobin disease- or HS-related genes, such as ANK1, SLC4A1, SPTA1, HBA1 and HBA2. The mutation was not included in the public database for the normal control population. The mutation was rated as pathogenic according to the American College of Medical Genetics and Genomics guidelines and the rating evidence was 'pathogenic very strong 1 + pathogenic moderate 2 + possibly pathogenic 4' (9). Sanger sequencing confirmed the presence of this mutation (Fig. 1). Finally, based on the clinical manifestations and family history, the patient was diagnosed with HS2 [Online Mendelian Inheritance in Man (OMIM) #616649].

Literature review. A total of 26 studies were reviewed, including 160 reported cases of HS in the Chinese population, of which 24 were neonatal cases (Tables SI and SII). The incidence of the ankyrin 1 (ANK1) mutation in the neonatal group (15/24, 63%) was higher than that in the non-neonatal group (58/136, 43%), while the incidence of the SPTB mutation was similar in the two groups. In addition, most of the mutations in the neonatal group were loss-of-function (LOF) mutations. For instance, the incidence of an LOF mutation in ANK1 was ~87% (13/15), while that in SPTB was 100% (8/8) (Fig. 2). This may indicate that patients with HS and LOF mutations are more likely to have a neonatal onset.

Discussion

HS is characterized by anemia, jaundice, progressive splenomegaly and reticulocytosis. Its clinical diagnosis is challenging, as the disease phenotype is highly variable. For instance, in patients with a mild phenotype and slight hemolysis, the condition is frequently ignored; however, when infection, fatigue and other specific factors aggravate hemolysis, subjects may develop symptoms similar to those of acute hemolytic anemia (10). The onset of HS in neonates is usually serious and the condition mainly manifests as anemia and hyperbilirubinemia. In general, neonatal cases with serious hemolysis and anemia symptoms require transfusion of suspended RBCs. However, with increasing neonatal age, the hematopoietic function of the bone marrow and the compensatory ability of the liver improve, which may alleviate the symptoms of anemia. To date, five pathological genes related to HS have been identified: ANK1 (OMIM #612641), SPTB (OMIM #182870), spectrum, α, erythrocytic 1 (OMIM #182860), solute carrier family 4, member 1 (SLC4A1; OMIM #109270) and erythrocyte membrane protein band 4.2 (OMIM #177070), among which ANK1 and SPTB defects are the main pathogenic factors of HS (3,11). In the present study, a patient with HS from a Han Chinese family was analyzed and it was indicated that the patient exhibited symptoms of jaundice, hyperbilirubinemia and anemia after natural delivery. Genetic testing revealed a novel frameshift mutation, p.Asp495fsTer78 in SPTB, which may lead to LOF gene mutation. Finally, the patient was diagnosed with HS2. In addition, the father of the patient carried the mutation and exhibited symptoms of anemia and splenomegaly during adolescence; however, the anemia symptoms subsided after splenectomy. The phenotypic variability of HS was further demonstrated by phenotypic differences among different family members.

SPTB is located on q23.3 of chromosome 14, which encodes the spectrin family β-subunit, and α-spectrin forms a tetramer α2β2 structure, which is an important part in forming the erythrocyte membrane skeleton network. When the function of the SPTB protein is altered, the cohesion of the membrane skeleton decreases, resulting in spherical changes in erythrocytes (2,3). The reported Chinese patients with HS-SPTB mainly harbored LOF mutations, such as nonsense and frameshift mutations (3,7,11). Wang *et al* (7) reported that the phenotypic severity of patients with HS had no significant correlation with pathogenic genes (ANK1, SPTB and SLC4A1) or mutation types. However, the retrospective analysis of the reported cases of HS in the Chinese population performed as part of the present study suggests that patients with neonatal onset more frequently harbor LOF mutations of pathogenic genes. However, whether a correlation exists between the mutation types and the age of onset of HS remains to be determined and requires further investigation through the analysis of more cases, particularly newborns, to establish the statistical significance of the results.

Table I. Laboratory indicators of the neonatal case of the present study.

Parameter	Reference range	Day 1	Day 2	Day 5
WBC, x10^9/l	3.50-9.50	21.50	16.03	16.16
Neutrophils, %	40.00-75.00	74.50	69.00	61.60
Lymphocytes, %	20.00-50.00	16.10	20.60	20.80
RBC, x10^{12}/l	6.00-7.00	4.43	4.31	3.65
Hemoglobin, g/l	170-200	147	142	120
Hematocrit, %	40.00-50.00	44.40	42.80	35.00
Mean corpuscular volume, fl	82.0-100.0	100.1	99.2	95.9
Mean corpuscular hemoglobin, pg	27.0-34.0	33.2	33.1	32.8
Mean corpuscular hemoglobin concentration, g/l	316-354	332	333	342
Reticulocytes, %	0.50-1.50	7.91	8.98	5.50
Reticulocyte count, x10^{12}/l	0.024-0.084	0.350	0.387	0.201
Total bilirubin, μg/l	5.0-21.0	206.1	218.9	222.6
Direct bilirubin, μg/l	0-6.8	0	0	28.5
Indirect bilirubin, μg/l	2.0-18.0	206.1	218.9	194.5
Aspartate aminotransferase, U/l	0-34	131	81	73
Alanine aminotransferase, U/l	0-49	18	13	16
γ-Glutamyl transpeptidase, U/l	0-73	243	213	204

WBC, white blood cell count; RBC, red blood cell count.

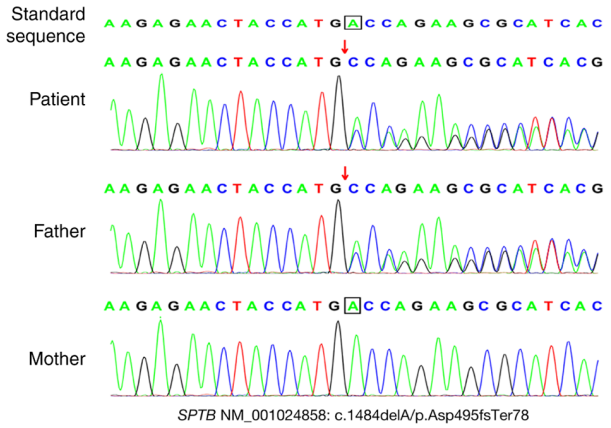

Figure 1. Sequencing analysis results highlight the c.1484delA/p.Asp495fsTer78 frameshift mutation in SPTB in the patient. The father was heterozygous for the frameshift mutation, whereas the mother was homozygous for the wild-type allele. SPTB, spectrum, β, erythrocytic.

As newborn HS cases usually have characteristics of non-specific diseases, such as jaundice and hyperbilirubinemia, clinical diagnosis is challenging. Furthermore, there are currently no diagnostic guidelines for newborns or infants with HS. Existing guidelines indicate that when the MCHC index of newborns is >36.0 g/dl, the sensitivity and specificity of identifying HS are as high as 82 and 98%, respectively (12). However, this index may not be applicable to the Chinese population. In the present study, previously reported data of 24 newborn cases of HS in the Chinese population were reviewed and 16 cases without detailed clinical data were excluded. Only one of the remaining eight newborn cases of HS had an MCHC index greater than the reference range (13). Of note, different researchers have used a variety of hematological indicators to upgrade the algorithm, which may improve the sensitivity and accuracy of HS identification. For instance, Tao *et al* (14) indicated through a cohort study that the sensitivity and identification of HS may be markedly improved when the mean sphered cell volume (MSCV) is less than the mean corpuscular volume (MCV), and Arora *et al* (15) determined that MCV-MSCV >10 fl may be used as the threshold range for diagnosing HS. However, neonatal cohort studies and statistical evidence for these new indicators for screening HS are lacking. In addition, in the present study, no MSCV index was found in reported neonatal cases, which may be related to the low availability of professional equipment for detecting this index. At present, only the Beckman Coulter blood analyzer is able to perform MSCV analysis (16).

Although key suggestive indicators for the screening and diagnosis of neonatal HS are still lacking, guidelines have listed the eosin-5'-maleimide binding test (EMABT) as one of the diagnostic methods for HS and confirmed its high sensitivity and specificity in numerous cohorts (17,18). However, EMABT may have limitations in neonatal patients. There are no obvious spherical RBCs in the peripheral blood images of certain neonatal patients, which interferes with clinical judgment (6,19). In addition, detection not only relies on the analysis of patients' fresh blood samples, but also the reproducibility of the results, which is affected by the stability and concentration of dyes. Therefore, laboratories need to have high standard requirements for detection timeliness and standardized management (20). In recent years, sequencing technology has been widely used in clinics due to its convenience, accuracy and high-throughput application, and it has an important role in HS diagnosis, differential diagnosis and genetic counseling. Since more than half of neonatal cases have a family history, the parents of newborns with hyperbilirubinemia and anemia symptoms should be asked for detailed family history information, which has a key role in early-intervention gene detection (21).

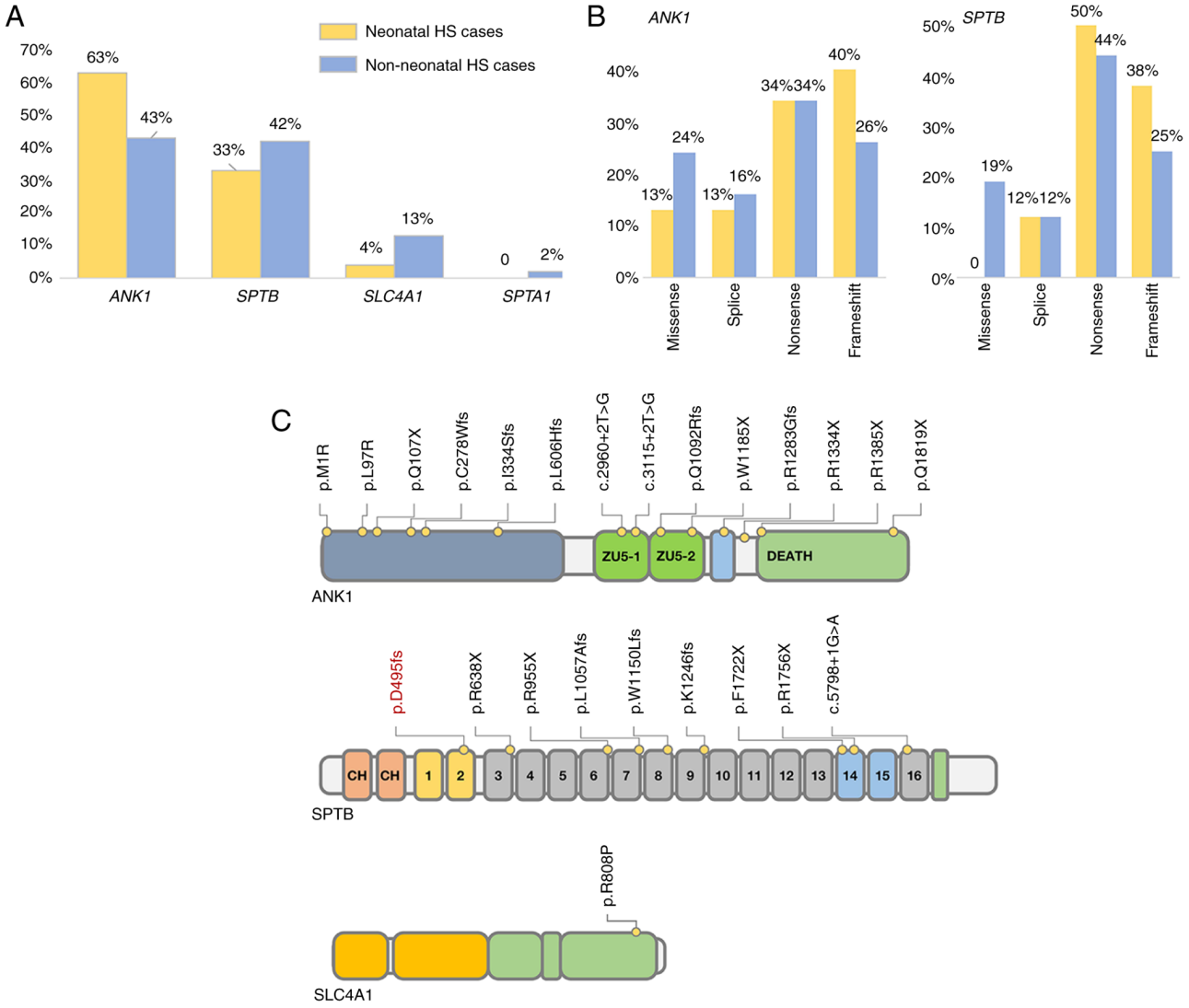

Figure 2. Genetic characteristics of the neonatal cases in China. (A) The incidence of ANK1 and SPTB mutations in neonatal and non-neonatal Chinese patients with HS. (B) Comparison of the frequency of varying loss-of-function mutations within ANK1 and SPTB in neonatal and non-neonatal patients with HS. (C) The reported mutation distribution of Chinese neonatal cases of HS; the position of the mutation investigated in this case is indicated in red. HS, hereditary spherocytosis; ANK1, ankyrin 1; SPTB, spectrum, β, erythrocytic; SLC4A1, solute carrier family 4, member 1; SPTA1, spectrum, α, erythrocytic 1.

In summary, the present study reported a case of neonatal HS. The patient had hyperbilirubinemia and anemia symptoms, but there were no abnormalities in MCHC or other indicators. Gene detection suggested a frameshift mutation in SPTB. A literature review suggested that Chinese neonatal cases are mainly caused by ANK1 defects, which have a higher incidence of LOF mutations than those in non-neonatal patients. In short, as neonatal HS has non-specific indicators, which challenges accurate diagnosis, it should be endeavored to actively improve the application value of gene detection technology in the diagnosis of HS.

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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

CX and NW designed the experiments. YW, DW and XZ collected and evaluated the clinical data. CX drafted the manuscript. CX and NW checked and approved the authenticity of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. This study was reviewed and approved by the ethics committee of the Fourth Affiliated

Hospital of Anhui Medical University (Hefei, China; approval no. PJ-YX2022-008).

Patient consent for publication

The parents of the patient provided written informed consent regarding the publication of the medical data and images of the case.

Competing interests

The authors declare that they have no competing interests.

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