A novel mutation in SPTA1 identified by whole exome sequencing in a Chinese family for hereditary elliptocytosis presenting with hyperbilirubinemia
A case report
Yaming Xi, MD, Lina Wang, MM, Pengpeng Zhang, BM, Mingfeng Jia, MM, Zijian Li, MD

Abstract
Rationale: Hereditary elliptocytosis is an inherited disorder characterized by the elliptical red blood cells (RBCs) on the peripheral blood smear and related hemolysis, mainly results from a heterozygous mutation in the genes that encode protein 4.1, \( \alpha \)-spectrin, \( \beta \)-spectrin. Mutations of SPTA1 are the most common.

Patient concerns: A 21-year-old female presented with left epigastric pain and jaundice with numerous elliptical RBCs on blood film. The family history review discovered jaundice in her sibling.

Diagnosis: A novel heterozygous mutation of SPTA1 was detected in the proband, her brother and father, c.7220_7221del:p.Tyr2407p in exon 52. Bioinformatics analysis indicated that this mutation was likely pathogenic and results in early termination of transcription and production of defective protein.

Interventions: The proband underwent splenectomy and cholecystectomy due to symptomatic splenomegaly and gallstone.

Outcomes: After surgery, the bilirubin levels decreased to normal (i.e., total bilirubin 16.4 \( \mu \)mol/L; indirect bilirubin 12.3 \( \mu \)mol/L), and the pain and discomfort in the upper abdomen relieved completely.

Lessons: We suggest that simultaneous whole exome sequencing of causative genes of all family members is a useful strategy to identify pathogenic variations for hereditary RBC membrane disorders, mainly in cases with an ambiguous phenotype.

Abbreviations: AD = autosomal dominant, HE = hereditary elliptocytosis, NGS = next-generation sequencing, RBC = red blood cell, WES = whole exome sequencing.

Keywords: exon52, hereditary elliptocytosis, pathogenic mutation, SPTA1 gene, whole exome sequencing.

1. Introduction
Hereditary elliptocytosis (HE) is a red blood cell (RBC) disorder with elliptically shaped erythrocytes in circulation and related hemolysis,[1,2] is common worldwide with a higher prevalence in malaria-endemic areas.[3] HE is typically inherited as an autosomal dominant disorder and the de novo mutations are rare.[3,4] Most HE is due to heterozygous mutations of genes that encode the \( \alpha \)-spectrin, \( \beta \)-spectrin, or protein 4.1. This disease occurs in about 1 per 3000 individuals worldwide and presents heterogeneously ranging from asymptomatic carriers to severe, life-threatening hemolytic anemia and splenomegaly.[3,5]

The mutated proteins in HE increase the fragility of the erythrocytic membrane skeleton. The erythrocyte spectrin is a scaffold protein composed of 2 subunits, \( \alpha \)-spectrin (encoded by SPTA1) and \( \beta \)-spectrin (encoded by SPTB), maintains the cellular shape, regulates the lateral mobility of integral membrane proteins, and provides structural support for the lipid bilayer.[4] Mutations in SPTA1 are the most common pathogenesis of HE, occurring in 65% of cases, followed by mutations in \( \beta \)-spectrin (30%) and protein 4.1 (5%).[6] Although a few mutations remote from the self-association site have been described, most mutations causing HE reside in the vicinity.[7] With discovered mutation increasing, there may be still many remnant unknown mutations can result in HE.[8]

Recent advances in next-generation sequencing (NGS) technology have led to a paradigm shift, leading the laboratory field of genetic testing away from Sanger sequencing.[9] The cost-effective, high-throughput NGS technology has resulted in the clinical application of unbiased genomic approaches, such as whole exome sequencing (WES),[10] allowing researchers to screen the wider panels of genes.[11] In this report, we identified a
novel SPTA1 mutation responsible for HE in a Chinese family using NGS, expanded the insights of molecular mechanisms, and proved it is reasonable to include this mutation in a genetic test for hereditary red cell membrane disorders.

2. Case presentation

A 21-year-old female was admitted to our hospital for continuous pain in the right upper abdomen and malaise in the left upper abdomen. Abdominal ultrasonography revealed cholecystolithiasis and mild splenomegaly. Marked elliptical RBCs were found on the blood film. A complete blood count (CBC) revealed mild anemia (white blood cell count, $3.88 \times 10^9$/L, hemoglobin level, 10.1 g/dL, and platelet count, $95 \times 10^9$/L, red cell distribution width, 56.3%). The Coomb’s test and osmotic fragility examination were negative. The serum biochemistry showed increased total bilirubin (126.6 μmol/L), indirect bilirubin (98.6 μmol/L), and lactate dehydrogenase (184 U/L). Bone marrow studies showed erythroid hyperplasia with the ratio of G:E of 0.32:1 (Fig. 1A) and elliptical RBCs taking 65% of red cells. Inquiry about family history disclosed that the proband’s younger brother had proved hyperbilirubinemia and that her younger brother (Fig. 1B, individual II-1) possesses similar traits such as high indirect bilirubin level in serum (Table 1).

To confirm the diagnosis of HE and identify the pathogenic gene, we did the WES for the proband, her sibling, and parents (Kindstar Global Medical Testing Center, Beijing, China). As a result, a heterozygous deletion of 2 nucleotides (i.e., AT) in exon 52 of the SPTA1 gene was identified as c.7220_7221del (p.Tyr2407*). Bioinformatic analysis indicated that the deletion leads to early termination of $\alpha$-spectrin at amino acid 2407 (the normal protein consists of 2419 amino acids), which may result in the production of a truncated or easily degraded protein. Then confirmation of this mutation was made by Sanger sequencing (Fig. 1C). Depth analysis of pedigree transmission showed that the proband, her brother, and father carry this mutation (Fig. 1B and C).

To treat gallstone and relieve hemolysis, the proband received cholecystectomy and splenectomy. After the surgery, the bilirubin levels decreased to normal (i.e., total bilirubin 16.4 μmol/L; indirect bilirubin 12.3 μmol/L), and the pain and uncomfortableness in the upper abdomen relieved completely.

**Figure 1.** (A) Bone marrow smears of the proband demonstrate elliptically shaped erythrocytes (magnification, ×1000). (B) Pedigree analysis of a Chinese hereditary elliptocytosis with a novel likely pathogenic mutation in SPTA1. Proband (indicated by the arrow), her sibling, and father were proved to carry the c.7220_7221del:p.Tyr2407* in the heterozygous state. (C) One heterozygous mutation of SPTA1 was identified by next-generation sequencing and confirmed by Sanger sequencing. A novel likely pathogenetic mutation annotated as c.7220_7221del:p.Tyr2407* in exon 52 of SPTA1.
3. Discussion

HE is typically inherited as an autosomal dominant disease with different characters ranging from asymptomatic carriers to patients with severe and even life-threatening hemolysis.[31] Neonatal jaundice, hemolytic anemia, and hydrops fetalis are also reported.[1] Although mutations of several genes that encoded the components of erythrocyte membrane skeleton can lead to HE, SPTA1 is the most prevalent.[6]

The SPTA1 gene locates in the q22-q23 region of chromosome 1 with a length of 80k nucleotides and contains 52 exons. The protein, defined as α-spectrin, is the essential subunit of spectrin that composes the erythrocyte membrane skeleton and contributes to resisting the shear stress in the circulation.[12] Thus, mutations in SPTA1 that influence the function of α-spectrin usually increase the fragility of erythrocyte and cause hemolysis. Most of the reported morbific mutations resident near the self-association site, substitute highly conserved amino acids and alter the interactions between spectrin subunits.[7,11] Although a few rare mutations of SPTA1 remote from the self-association site have been reported,[4,12,14,15] mutations involved in the last exon of SPTA1, exon52, have not been reported.

By NGS sequencing, we found a novel mutation in the exon52 of SPTA1 gene related to HE in a Chinese family, c.7220_7221del(p.Tyr2407*), that produces a truncated α-spectrin protein without the 12 residues of c-terminal. However, the major drawback of current NGS applications is the difficulty in determining the pathogenicity of the identified variants.[4] One of the ways to overcome this limitation is the simultaneous evaluation of all family members, allowing one to establish the inheritance pattern of the identified variants and thus to understand its pathogenic role.[4] Thus, the identification by WES and pedigree transmission indicated together that this mutation is pathogenic and transmitted from the father (Fig. 1B and C). Moreover, this find indicated that the c-terminal is also critical for the function of α-spectrin. It is reasonable to include this mutation in diagnostic test and genetic counseling for hereditary red cell membrane disorders.

4. Conclusion

In summary, we identified a novel mutation (p.Tyr2407) in the exon52 of SPTA1 using NGS in a Chinese family that can cause HE. This study expands the insights of molecular mechanisms and presents a novel marker for diagnostic test and genetic counseling for hereditary red cell membrane disorders.

Author contributions

Data curation: Lina Wang, Mingfeng Jia.
Formal analysis: Yaming Xi, Lina Wang, Zijian Li.
Investigation: Yaming Xi, Pengpeng Zhang.
Project administration: Yaming Xi, Lina Wang, Pengpeng Zhang.
Resources: Mingfeng Jia.
Supervision: Zijian Li.
Validation: Zijian Li.
Visualization: Zijian Li.
Writing – original draft: Yaming Xi, Lina Wang.
Writing – review and editing: Zijian Li.

Zijian Li orcid: 0000-0002-6796-4176.

Table 1

| Characteristics | Father (I-1) | Brother (II-1) | Proband (II-2) |
|-----------------|-------------|---------------|---------------|
| Sex/Age, y      | M/52        | M/22          | F/21          |
| RBC, ×10^12/μL | 4.24        | 4.48          | 3.39          |
| Hemoglobin, g/dL| 13.1        | 14.4          | 10.1          |
| MCV, fl (80–100)| 90.6        | 89.7          | 85.3          |
| MCH, pg (27–31)| 30.9        | 32.1          | 29.8          |
| MCHC, % (32–36)| 34.1        | 35.8          | 34.9          |
| RDW, % (59–46) | 41.4        | 47.5          | 56.3          |
| Reticulocytes, % (0.5–2)| 3.6 | 4.8 | 13.7 |
| Erythropoietin, MNU/mL | Not done | Not done | Normal |
| Osmotic fragility | Not done | Not done | Normal |
| LDH, IU/L (135–250)| 202 | Not done | 184 |
| Total bilirubin, umol/L (0–30.1)| 29 | 63.1 | 126.6 |
| Indirect bilirubin, umol/L (0–19)| 24.8 | 54.1 | 98.6 |
| Coombs (direct/indirect) | Not done | Not done | Negative/Negative |
| Splenomegaly | Negative | Negative | Positive |

LDH = lactate dehydrogenase, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, RBC = red blood cell, RDW = red cell distribution width.

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