Clinical Case Report

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A novel mutation in *SPTA1* identified by whole exome sequencing in a Chinese family for hereditary elliptocytosis presenting with hyperbilirubinemia

A case report

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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, *α-spectrin*, *β-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.Tyr2407* 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 μmol/L; indirect bilirubin 12.3 μmol/L), and the pain and uncomfortableness 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 pathogenetic mutations 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 *α-spectrin*, *β-spectrin*, or protein 4.1. This disease occurs in about 1 per 3000 individuals worldwide and presents heterogeneously ranging from asymptomatic carries to severe, life-threatening hemolytic anemia and splenomegaly.[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, *α-spectrin* (encoded by *SPTA1*) and *β-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 β-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 increaseing, 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

Editor: N/A.

YX and LW equally contributed to this work.

The authors have no funding and conflicts of interest to disclose.

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Medicine (2019) 98:22(e15800)

Received: 27 January 2019 / Received in final form: 13 April 2019 / Accepted: 29 April 2019

http://dx.doi.org/10.1097/MD.0000000000015800

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, 3.88×10^9/L, hemoglobin level, 10.1 g/dL, and platelet count, 95×10^9/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 *α-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.

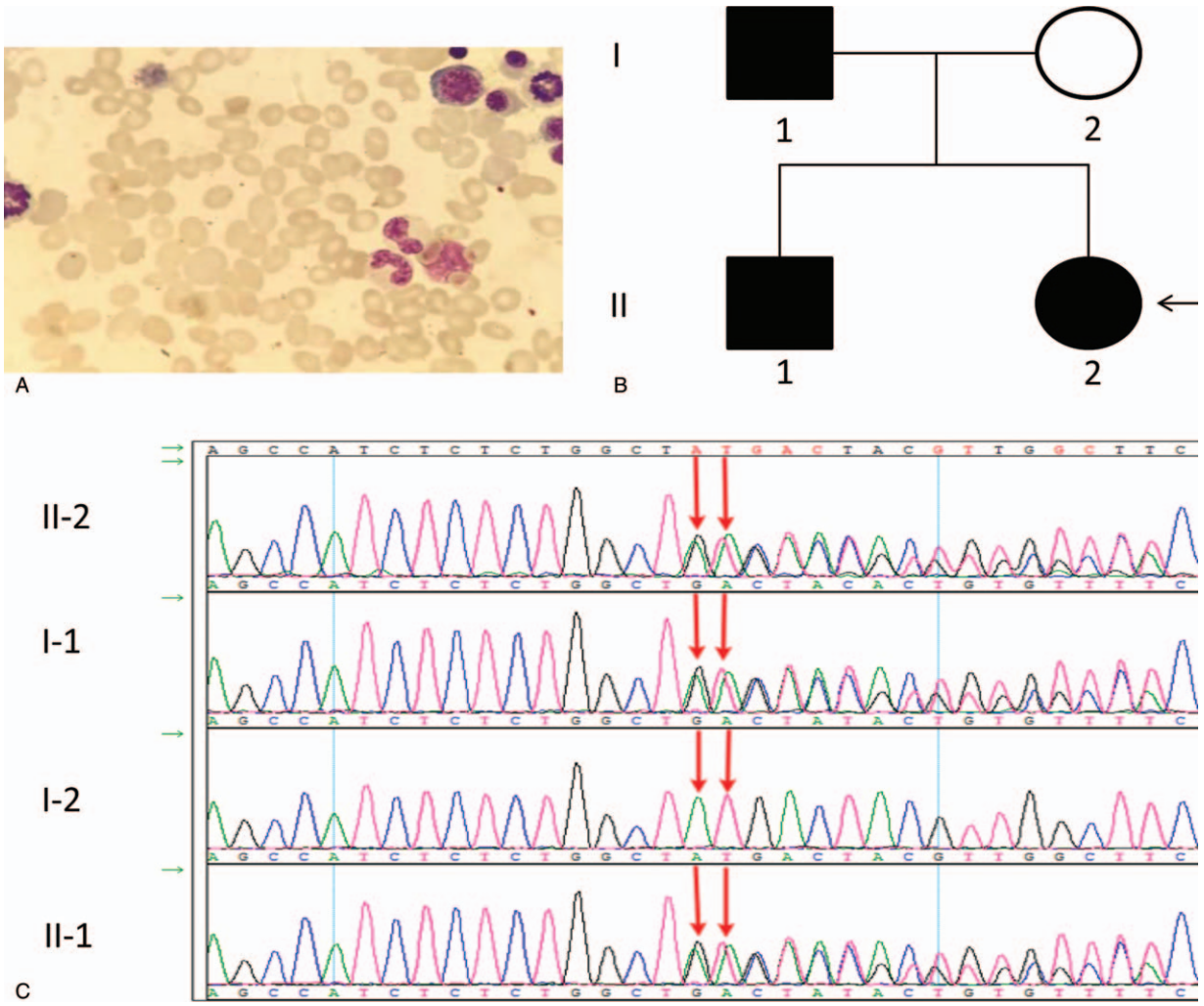

Figure 1. (A) Bone marrow smears of the proband demonstrate elliptically shaped erythrocytes (magnification, ×1000). (B) Pedigree analysis of a Chinese hereditary elliptocytosis with a novel likely pathogenic mutation in *SPTA1*. Proband (indicated by the arrow), her sibling, and father were proved to 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*.

Table 1

Laboratory findings and clinical traits of the patients.

Characteristics	Father (I-1)	Brother (II-1)	Proband (II-2)
Sex/Age, y	M/52	M/22	F/21
RBC, $\times 10^{12}$/L (4–5.5)	4.24	4.48	3.39
Hemoglobin, g/dL (12–16)	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, % (39–46)	41.4	47.5	56.3
Reticulocytes, % (0.5–2)	3.6	4.8	13.7
Erythropoietin, MIU/mL	Not done	Not done	66.1
Osmotic fragility	Not done	Not done	Normal
LDH, IU/L (135–250)	202	Not done	184
Total bilirubin, umol/L (9.1–30.1)	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	Postive

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.

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-threating hemolysis.[5] 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.[7,13] Although a few rare mutations of *SPTA1* remote from the self-association site have been reported,[6,12,14,15] 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 pathogenetic 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

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