Diagnosis of Hereditary Spherocytosis and Secondary Hemochromatosis in a Patient with Jaundice

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Hereditary spherocytosis (HS) is a type of hemolytic anemia resulting from hereditary defects in the erythrocyte membrane. The main clinical features are anemia, jaundice, splenomegaly, gallstones, visible spherical red blood cells (RBC) in the peripheral blood, and increased RBC osmotic fragility. The pathological cause of HS is a genetic mutation that leads to an RBC membrane protein deficiency, increased cell permeability, decreased deformability, and increased damage to the spleen. The genes involved are ANK1, SLC4A1, SPTA1, SPTB, EPB42, and EPB41; ANK1 mutations are the most common [1, 2]. Splenectomy is an effective and safe treatment for HS symptom relief, especially in children [3, 4]. With sporadic treatment, the misdiagnosis and missed diagnosis rates are high [5]. The incidence of HS is reportedly much higher than the clinical diagnosis rate [6]. Morton et al. [7] studied the genetic characteristics of HS and found that while HS can occur in all ethnic groups, its genetic frequency remains unknown. Approximately 75% of cases are autosomal dominant, and the ANK1 gene is most commonly mutated. The other 25% cases are autosomal recessive; in these cases, although there may be no family history, other relatives may also have gene mutations, phenotypic variations, and autosomal recessive inheritance [8].

According to the 2011 guidelines for HS diagnosis and management of the General Haematology Task Force of the British Committee for Standards in Haematology, HS can be diagnosed without further tests if a patient has the combination of a family history of HS and typical HS clinical manifestations and blood parameters. Currently, there are no laboratory tests for an HS diagnosis alone; the combination of the eosin-maleimide test (EMA) with the acidified glycerol lysis test (AGLT) is the first choice, and it has a sensitivity of almost 100%. The sensitivity of the combination of the erythrocyte osmotic fragility test (OFT) with AGLT is 97%, while that of the combination of the EMA and the OFT is 95%. If the patient's blood smear results are positive, the family members will be recommended to undergo the OFT and EMA, too [9]. Patients with normal blood smears from the parents will be screened for molecular defects, which may indicate recessive inheritance resulting from new mutations in the SPTB and ANK1 genes. Further analysis of the SPTB and ANK1 genes of patients and their families may reveal new single-allele gene expression as the cause of the disease [10].

Here, we found a novel pathogenic mutation in ANK1. We present the case of a 51-year-old man repeatedly experiencing

fatigue and yellow urine for 10 years. The patient had undergone cholecystectomy. He had suffered hyperbilirubinemia and had undergone a bone marrow puncture when he was an infant (data not available). There is no similar history in his family. Laboratory tested revealed the following: white blood cell count, $6.14 \times 10^9/L$; RBC count, $3.39 \times 10^{12}/L$; hemoglobin, 99 g/L; mean corpuscular volume, 83.8 fL; platelet count, $145 \times 10^9/L$; reticulocyte count, 9.41%; total bilirubin, 96.2 $\mu\text{mol}/L$ (reference value: 1.7–25.6 $\mu\text{mol}/L$); indirect bilirubin, 79.7 $\mu\text{mol}/L$ (reference value: 0.1–21 $\mu\text{mol}/L$); total iron binding capacity, 51.4 $\mu\text{mol}/L$; iron, 12.5 $\mu\text{mol}/L$; transferrin saturation, 24.3%; ferritin, 523.41 $\mu\text{g}/L$; copper protein, 282 mg/L. B ultrasound revealed hepatomegaly and splenomegaly. Peripheral blood smear showed 40% spherical RBCs by microscopy. The erythrocyte OFT was normal. For differential diagnosis, the patient underwent the following tests: sucrose hemolysis, serum acidification hemolysis, Coombs, alkali denaturation, hemoglobin A₂, hemoglobin electrophoresis, hemoglobin H inclusion body, modified globin body, isopropyl alcohol, methemoglobin reduction, glucose 6-phosphate dehydrogenase fluorescent spot, and glucose 6-phosphate dehydrogenase activity tests, all of which yielded normal results.

Table 1. Gene mutations

Gene	Nucleotide change	Amino acid change	Exon	Genotype	Position	dbSNP	Predicted effect
ANK1	c.2803 C→T	p.R935X	26	het-var	chr8:41554038		nonsense/damaging
ANK1	c.5265 G→A	p.V1755V	39	hom-var	chr8:41525914	rs750625	synonymous
ANK1	c.315 C→T	p.N105N	74	het-var	chr8:41585438	rs2304871	synonymous
SPTA1	c.7095 A→G	p.A2365A	51	het-var	chr1:158582646	rs2251969	synonymous
SPTA1	c.6794 T→C	p.I2265T	49	het-var	chr1:158584091	rs952094	missense/benign
SPTA1	c.5958 C→T	p.P1986P	43	het-var	chr1:158592935	rs3753068	synonymous
SPTA1	c.5572 C→G	p.L1858V	40	het-var	chr1:158597507	rs3737515	missense/benign
SPTA1	c.5292 C→A	p.A1764A	37	het-var	chr1:158606449	rs3738791	synonymous
SPTA1	c.4702 T→C	p.C1568R	33	het-var	chr1:158612236	rs863931	missense/benign
SPTA1	c.3487 T→G	p.S1163A	25	hom-var	chr1:158619728	rs2482965	missense/benign
SPTA1	c.2874 G→A	p.L958L	20	het-var	chr1:158626378	rs857691	synonymous
SPTA1	c.2493 T→G	p.L831L	18	het-var	chr1:158631171	rs2518493	synonymous
SPTA1	c.942 T→A	p.A314A	77	hom-var	chr1:158647495	rs325996	synonymous
SPTA1	c.915 T→C	p.S305S	77	het-var	chr1:158647552	rs703121	synonymous
SPTA1	c.126 C→T	p.V42V	72	hom-var	chr1:158655036	rs435080	synonymous
SPTB	c.4860 T→C	p.I1620I	23	hom-var	chr14:65241228	rs229592	synonymous
SPTB	c.4641 G→A	p.A1547A	22	het-var	chr14:65242044	rs184528	synonymous
SPTB	c.1316 G→A	p.S439N	10	het-var	chr14:65263300	rs229587	missense/benign
SPTB	c.1269 G→A	p.L423L	10	het-var	chr14:65263347	rs229586	synonymous

Therefore, the diagnosis of HS was confirmed. In addition, upper abdominal nuclear magnetic resonance (NMR) spectroscopy revealed iron deposition in the liver and spleen. A liver biopsy indicated liver hemosiderosis with mild fibrosis, and, in view of the normal transferrin saturation, he was diagnosed with secondary hemochromatosis.

Then, we detected 6 exons of HS-related genes through next-generation high-throughput sequencing. Analyzing the function of and variation in each gene, it was found that the ANK1, SPTA1, and SPTB genes were mutants (Table 1) without SLC4A1, EPB42, or EPB41 gene mutations. According to the classification of genetic variants published by the American College of Medical Genetics and Genomics (ACMG), ANK1 (MN_000037.3) exon 26: c.2803C→T was suspected pathogenic mutation, which was ultimately verified by Sanger sequencing (Fig. 1). His father had passed away and had no clinical manifestations while alive, and the blood smear results of the patient's mother, son, and daughter were all normal. Therefore, it was considered a novel nonsense mutation.

In summary, as HS is prone to be misdiagnosed and missed altogether, relevant medical projects should be performed thoroughly in medical institutions. Clinicians should also raise the awareness of HS

Fig. 1. Sanger sequencing results. **a** The 935th amino acid changed from arginine to a stop codon. **b, c** Sanger sequencing results of the reference base sequence (**b**) and the patient's base sequence (**c**). **b** Arrow indicates the ANK1 gene c.2803 site, which was normal. **c** Arrow indicates the heterozygous mutation in the ANK1 gene: c.2803C→T.

and take it into account, especially in cases of unexplained jaundice. Here, we diagnosed a patient with HS and secondary hemochromatosis, and we found a novel nonsense mutation. Moreover, we used a new technique, upper abdominal NMR spec-

troscopy, to assess the iron content of the liver, which could help predicting the prognosis. However, further clinical studies still need to be performed to explore the genetic types and hereditary characteristics of HS.

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