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CASE REPORT

A Case of Adult Hereditary Spherocytosis Concomitant with Gilbert Syndrome Caused by Mutations in SPTB and UGT1A1

Yang Gou[1,2], Ping Wang[1,2], Wucheng Yang[1,2], Yimei Feng[1,2], Xiangui Peng[1,2], Hong Liu[1,2], Shuiqing Liu[1,2], Xi Zhang[1,2]

[1]Medical Center of Hematology, Xinqiao Hospital of Army Medical University, Chongqing, 400037, People's Republic of China; [2]Chongqing Key Laboratory of Hematology and Microenvironment, Chongqing, 400037, People's Republic of China

Correspondence: Hong Liu; Shuiqing Liu, Email 1241580141@qq.com; liushuiqing062@tmmu.edu.cn

Abstract: Hereditary spherocytosis (HS) is the most common hereditary hemolytic disease with defects in red blood cells (RBC) membrane proteins caused by mutations in membrane protein genes, like SPTB, SPTA1 and ANK1. Gilbert syndrome (GS) is a disease characterized by a mild deficiency of uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1) enzyme activity and unconjugated hyperbilirubinemia, largely caused by UGT1A1 mutations. The two inherited diseases HS and GS are rarely occurred in the same patient and are easy to be misdiagnosed, resulting in excessive diagnosis and treatment. Here, we report a rare case of HS combined with GS due to mutations in the SPTB and UGT1A1 genes. A 50-year-old man who had an over 40-year history of jaundice was admitted to our hospital owing to fatigue and fever. His blood analysis showed low hemoglobin (74 g/L), high reticulocyte (23.5%) and high serum bilirubin (65 μmol/L); abdominal ultrasound revealed calculous cholecystitis and splenomegaly. Considering a possible diagnosis of hemolytic anemia, further examinations showed 42% spherocytes in blood smears and high erythroid lineage hyperplasia in bone marrow. Subsequently, 151 jaundice-related genes panel sequencing was done and results showed SPTB p.N1260fs and UGT1A1 p.G71R mutations. Then the patient was diagnosed with HS complicated with GS. Anti-infection and supportive treatments were providing to the patient, while infection removed, the hemoglobin recovered to normal, and no additional treatment was given. These findings of this report indicate that patients who are considered hemolytic anemia presenting with jaundice and anemia, genetic testing is a crucial method for the final diagnosis and bilirubin metabolic disease should also be concerned.

Keywords: hereditary spherocytosis, Gilbert syndrome, SPTB, UGT1A1

Introduction

Hereditary spherocytosis (HS) is a common heterogeneous disease with defects in red blood cells (RBC) membrane proteins, which destroy the vertical connection between the cytoskeleton and phospholipid bilayer, resulting in RBC losing their normal biconcave shape and plasticity, and becoming spherical.[1] It is identified by the presence of spherical erythrocytes on the peripheral blood smears and has a variety of clinical manifestations, such as anemia, splenomegaly, and jaundice.[1] The overall prevalence of HS in China was estimated to be 1.27 and 1.49 in 100,000 for males and females, respectively.[2] However, patients with HS, autoimmune hemolytic anemia, or thalassemia have similar clinical picture. How to discriminate them rapidly and accurately is a crucial clinical issue. When elevated spherocytes and reticulocytes and decreased mean reticulocyte volume were found in patients with anemia and jaundice, the HS may be concerned.[3] With the assistance of sequencing technologies in clinical diagnosis, more and more novel mutant genes have been recognized to be associated with HS, like beta-spectrin (SPTB), ankyrin 1 (ANK1), alpha spectrin (SPTA1), non-erythrocytic alpha-II-spectrin (SPTAN1), protein 4.2 (EPB42), and red blood cell anion exchanger 1 (also known as band 3, AE1 or SLC4A1).[4] Mutation of just one HS-related gene can result in membrane protein defects, leading to HS occurrence.

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Gilbert syndrome (GS) refers to an autosomal recessive genetic disorder characterized by a deficiency of uridine diphosphate-glucuronosyltransferase 1A1 (UGT1A1) enzyme activity and unconjugated hyperbilirubinemia.[5] This disease is estimated to be 3%–7% in the general population and mostly occurs in males.[6] Patients with GS often present with mild intermittent jaundice. GS is largely caused by UGT1A1 mutations. There are three UGT1A1 related mutations commonly seen in clinical practice. First, the increased number of TA repeats in the promoter region of UGT1A1 (normal is A(TA)6TAA; abnormal is usually A(TA)7TAA, aslo named polymorphism UGT1A1*28) have a very high prevalence in the Western populations, while have a low incidence in Asian populations.[7] Second, the existence of variant c.-3279T>G (rs4124874) in PBREM, which is located in the enhancer region approximately 3.2 kb upstream of the UGT1A1 transcription start site, is common in China with an incidence rate of 36%.[8] Third, point mutation on the exon region of UGT1A1 p.G71R mutation is the most frequently-occurring type, including homozygous mutations and heterozygous mutations.[7] And UGT1A1 enzyme activity in patients with GS can reduce to about 30% to 60% of the normal activity, respectively, according to heterozygous or homozygous alterations.[9] The total bilirubin level can reach to 90μmol/L in GS, and higher bilirubin levels can be seen in Crigler-Najjar syndrome type I and type II which is an another hereditary hyperbilirubinemias disease and is usually caused by UGT1A1 alterations. While the UGT1A1 enzyme is completely non-functional in the type I, and is decreased in the type II.[7]

The two inherited diseases HS and GS are rarely occurred in the same patient and are easy to be misdiagnosed. Once GS has been associated with some hemolytic diseases (including HS), the bilirubin level can be abnormally increased and the risk of biliary calculosis is very high.[7] Herein, we reported a rare case of HS combined HS with GS caused by mutations in the SPTB and UGT1A1 genes. We also summarized the specific clinical manifestations and various laboratory test results. We hope this report will help clinicians to better understand the jaundice-associated diseases and avoid excessive diagnosis and treatment.

Case Report

A 50-year-old man who presented with yellow staining of sclera and skin over 40 years and suffered from fatigue and fever was admitted to our hospital on 12 May 2023. The patient had visited another hospital (Tongren People's Hospital, Guizhou Province, China) three days prior and physical examination revealed mild splenomegaly and did not found any systemic superficial lymph node enlargement. His blood analysis tests were as follows: white blood cell count, 15.1×10^9/L (reference range 4–10×10^9/L); neutrophil percentage, 75.9% (reference range 50–70%); hemoglobin, 65g/L (reference range 120–160 g/L); platelet count, 223×10^9/L (reference range 100–350×10^9/L); reticulocyte, 14.7% (reference range 0.8–2%); mean corpuscular volume, 85.9 fL (reference range 80–100 fL); folate 3.0 ng/mL (reference range >5.9 ng/mL), vitamin B12, 157 pg/mL (reference range 180–914 pg/mL); ferritin, 1422 ng/mL (reference range 24–336 ng/mL); serum iron, 13.9 μmol/L (reference range 11.5–31.3 μmol/L); total iron-binding capacity, 29 μmol/L (reference range 50–77 μmol/L); total serum bilirubin, 65 μmol/L (reference range 3–20 μmol/L); direct bilirubin, 22 μmol/L (reference range 0–7 μmol/L). Abdominal ultrasound revealed calculous cholecystitis and the echoes of the liver showed dense thickening. Besides, the spleen thickness was approximately 5.4 cm with a diameter of 15.1cm. Based on the above findings, piperacillin/tazobactam was administered for anti-infection treatment and folic acid and mecobalamin tablet for anemia treatment. Subsequently, this patient was presented to our outpatient clinic for the first time on 12 May 2023. Blood test results were as follows: white blood cell count, 14.8×10^9/L (reference range 3.5–9.5×10^9/L); hemoglobin, 74 g/L (reference range 130–175 g/L); platelet count, 235×10^9/L (reference range 100–300×10^9/L); reticulocyte, 23.5% (reference range 0.5–1.5%); mean corpuscular volume, 97.5fL (reference range 80–100 fL); folate 6.7 ng/mL (reference range 3–20 ng/mL), vitamin B12 217 pg/mL (reference range 180–900 pg/mL); ferritin, 820 ng/mL (reference range 15–200 ng/mL); total iron-binding capacity, 38 μmol/L (reference range 50–75 μmol/L). The two hospitals showed similar clinical examination results.

To further investigate the underlying etiology of anemia, we also performed a series of tests. The direct antiglobulin test and antibody screen were both negative, not supporting autoimmune hemolysis. Ham and Hemolysis test represented negative results, not supporting paroxysmal nocturnal hemoglobinuria. Methemoglobin reduction test was also negative, not supporting haemolytic anaemia due to Glucose-6-phosphate dehydrogenase deficiency. We also conducted red blood cell fragility, however, the specimen was hemolyzed and the results were not available. Peripheral blood smears showed

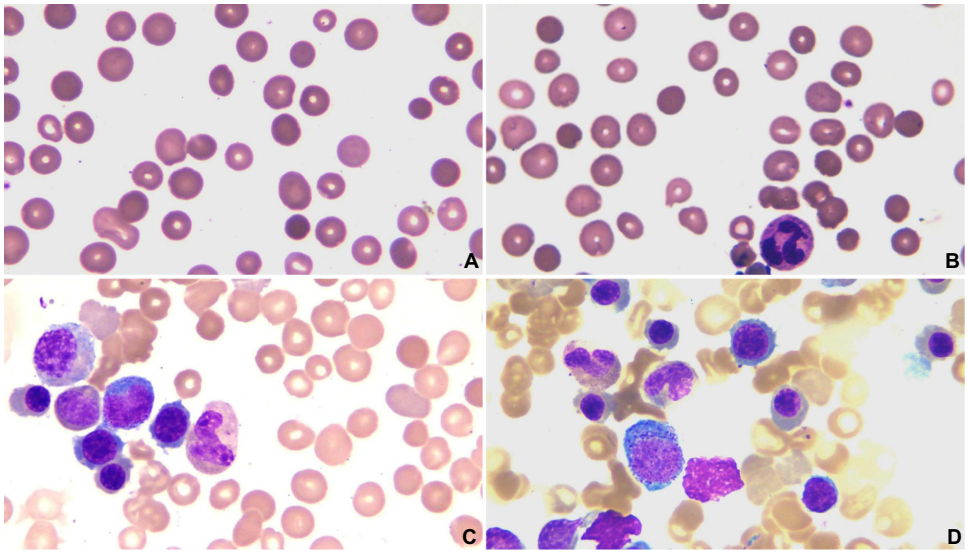

Figure 1 Peripheral blood and bone marrow smear of the patient. (**A**) and (**B**) Peripheral blood smear images (Wright's stain, 100×). (**C**) and (**D**) Bone marrow smear images (Wright's stain, 100×).

42% spherocytes. Bone marrow smears indicated that significant active bone marrow hyperplasia, of which, granulocytic lineage account for 29.5%, erythroid lineage account for 60.5%, and granulocyte/erythrocyte ratio was 0.49:1 (Figure 1). Moreover, nucleated cells appeared grossly normal in shape and bone marrow iron stain revealed extracellular iron (+++), sideroblasts 60%, suggesting an iron overload condition. So the diagnosis is locked to hemolytic anemia caused by HS. Subsequently, targeted sequencing of the exon regions of 151 jaundice related genes in genomic DNA extracted from the patient's peripheral blood were performed by library-based sequencing platform ABI 3730 automated DNA sequencer (Applied Biosystems, USA). And results showed SPTB p.N1260fs and UGT1A1 p.G71R mutations (Table 1). Other examinations were done when the patient accepted the hepatobiliary surgery. The average plain MRI T2 scan value of liver, bile, pancreas, spleen showed 5.22 ms, suggesting mild iron deposition in hepatic. The pathology of liver biopsy indicated cholestatic liver injury, extra and intrahepatic bile ducts obstruction, hepatic iron deposition (grade 2-3, corresponding to modified Scheuer score G2S1). Based on the above test results, the patient was diagnosed with HS complicated with GS. The patient was given anti-infection and supportive therapy. After resolution of the infection, the hemoglobin returned to the normal level and the patient did not receive any additional treatment. In addition, the patient did not state a similar family history. Regretfully, genetic testing was not done in other family member. The diagnostic process of this patient was shown in the Figure 2.

Discussion

Jaundice is the yellowish discolouration of the skin, sclera and mucous membranes due to the elevated bilirubin in the body (hyperbilirubinemia). Hemolysis, cholestasis, liver cell damage and liver dysfunction can cause jaundice. Hemolytic anemia usually presents with anemia, jaundice and splenomegaly. Therefore, it is easy to distinguish between hemolytic anemia and jaundice in most cases. However, the underlying causative is often difficult to pinpoint, especially inherited disease without a clear family history. So overdiagnosed and overtreated frequently occurred in these patients. Fortunately, gene mutation analysis is helpful to diagnose these diseases. The patient in this report presented with p.

Table 1 Gene Mutations Detected in the Next-Generation Target Sequencing

Gene	Chromosome	Position	ID	Mutation	Pattern
UGT1A1	Chr2:234669144	Exon1	NM_000463.2	c. 211G>A, p. Gly71Arg	Heterozygous
SPTB	Chr14:65252331	Exon18	NM_001355436.1	c. 3779delA, p. Asn1260fs	Heterozygous

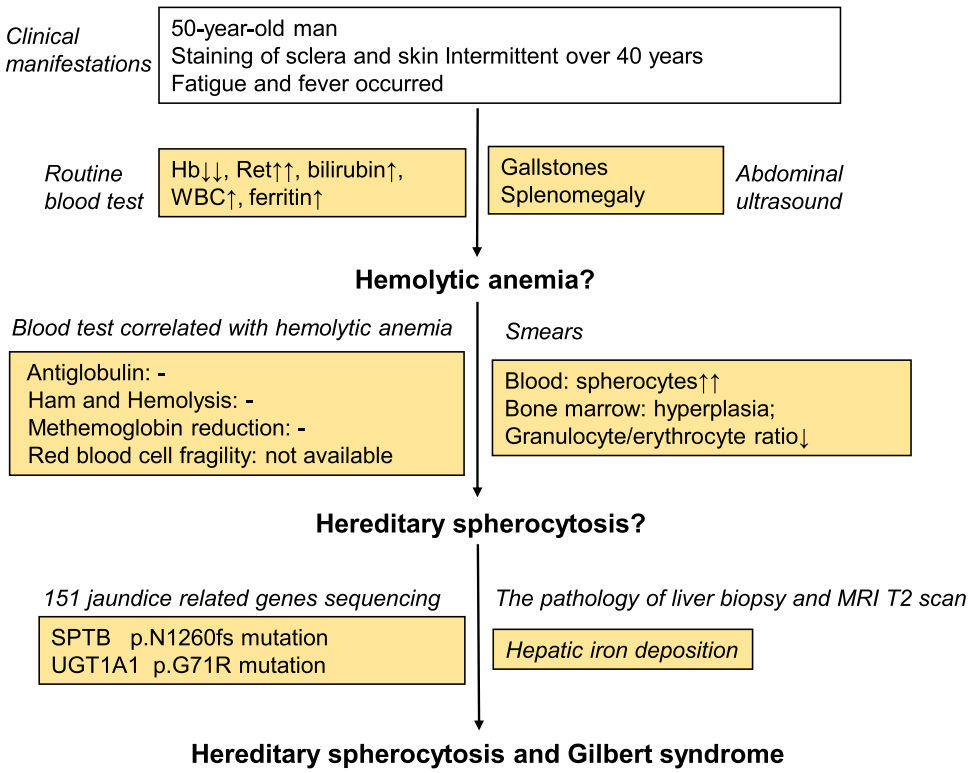

Figure 2 Flow chart showing the diagnostic process for the patient with hereditary spherocytosis and Gilbert syndrome.

G71R heterozygous mutations, leading to an approximate 40% decrease in enzymatic activity of UGT1A1, thereby developing serum unconjugated hyperbilirubinemia. As he also had HS, the level of unconjugated hyperbilirubinemia in serum was further elevated. Regarding the source of the patient's mutation sites, it may be from the patient's father, mother, or combined with an embryonic gene mutation. Regretfully, genetic testing was not done in his family member, and we were unable to identify the source of the patient's mutation sites. But his parents both showed no clinical manifestations, implying that his parents may each carry one mutation, and this do not cause disease. Subsequently, we summarized 17 HS concomitant with GS cases during 2000–2023 reported in literature. All cases had alterations in UGT1A1 gene, but only 3 gene mutations (ANK1/SLC4A1/SPTA1) were reported associated with HS (Table 2).[6,10–20] None of cases were caused by SPTB and UGT1A1 mutations simultaneously.[6,10–20] Among them, 12 patients were male and 5 patients were female with an age range of 0–58 years at diagnosis; hemoglobin levels and percentage of reticulocytes ranged from 95 to 152 g/L, 3%-25.7%, respectively; total serum bilirubin was significantly elevated and the number of spherocytes was increased in all cases; 10/17 (58.8%) cases had gallstones or splenomegaly; 4/17 (23.5%) patients underwent splenectomy, the decreased bilirubin levels were found in 2 patients and reduced reticulocytes and raised hemoglobin levels were found in another 2 patients after splenectomy; 3/17 (17.6%) patients underwent cholecystectomy and 2/17 (11.8%) patients were given phenobarbitones.[6,10–20] When patients have both HS and GS disease, the risk of gallstone will increase from 15% to 50%.[21] In our case, patient had gallstones with gastritis, cholecystectomy should be performed when its necessary. For patients with high bilirubin levels, phenobarbital treatment can decrease bilirubin at an appropriate level. Splenectomy is effective to improve hemolysis, anemia, and hyperbilirubinemia, but patients are more likely to suffer infection, thromboembolism, and cardiovascular. Therefore, splenectomy is not recommended when patients present with mild-moderate anaemia, but it is available for severely anemic and transfusion-dependent patients.[22]

In this case, his hemoglobin was less than 80 g/L, the percentage of reticulocytes was greater than 10%, splenomegaly enlargement was more than 4.7 cm, which met the criteria for severe HS. Thus, splenectomy can be considered to prevent the disease from further worsening in this case. Additionally, iron removal treatment is required owing to the liver iron

Table 2 Clinical Information of 17 Cases with Combined Hereditary Spherocytosis and Gilbert Syndrome Reported in the Literature

Reference	Year	Gender	Age of Diagnosis	Hemoglobin (g/L)	Reticulocytes (%)	Gallstones	Splenomegaly	Gene Mutation	Treatment
[6]	2014	Boy	12	133	17.5	Yes	Yes (17cm)	UGT1A1 (A(TA)7TAA, homozygous)	Splenectomy, cholecystectomy
[10]	2007	Girl	10	128	12.6	Yes	Yes	UGT1A1 (A(TA)7TAA, heterozygous)	Splenectomy
[11]	2008	Boy	17	80	4.5	Yes	Yes	UGT1A1 (A(TA)7TAA, homozygous)	Splenectomy, cholecystectomy
[12]	2010	Man	28	110	6.3	Yes	Yes	UGT1A1 (A(TA)7TAA; p.G71R)	Considered for splenectomy
[13]	2011	Girl	0 (New-born)	80	4.9	No	No	UGT1A1 (A(TA)7TAA, homozygous)	Phenobarbitone, triple phototherapy
[14]	2011	Woman	25	124	NA	Yes	NA	UGT1A1 (p.G71R; Y486D)	Splenectomy
[15]	2012	Boy	10	80	4.5	Yes	Yes	UGT1A1 (A(TA)7TAA; p.G71R)	Not known
[16]	2013	Man	41	130	NA	Yes	Yes (13 cm)	UGT1A1(A(TA)7TAA; p.G71R; c.3279T>G)	Refused for cholecystectomy
[17]	2017	Man	19	138	3.0	Yes	Yes	UGT1A1 (A(TA)7TAA, heterozygous; p.G71R, heterozygous)	Not known
[18]	2018	Girl	21-month	84	25.7	No	No	UGT1A1 (A(TA)7TAA, homozygous)	Phenobarbitone
[19]	2020	Man (5 cases) Woman(1 case)	25–58	115–158	NA	Yes (2 cases)	Yes (3 cases)	UGT1A1; ANK1/SLC4A1	No treatment
[20]	2023	Boy	15	93	7.9	No	No	UGT1A1 (p.G71R); SPTA1	Folic acid

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deposition caused by long-term hemolysis. His fever was accompanied by asthenia and weakness after infection. Studies have shown that viral or bacterial infections can aggravate hemolysis and lead to a reduction in hemoglobin level.[23] We observed that the symptom of hemolysis was minimized and hemoglobin level was elevated in patient after the signs of infection disappeared. Therefore, hemolytic patients need to enhance immunity to avoid infection. Additionally, transplantation is another choice in the patients with severe anemia with genetic alterations.[24]

Overall, HS associated with GS is a rare condition and is easily misdiagnosed, resulting in excessive diagnosis and treatment. As for patients who are considered hemolytic anemia presenting with jaundice and anemia, genetic testing is a crucial method for the final diagnosis and bilirubin metabolic disease should also be concerned, especially the patient tested negative for the antiglobulin test both direct and indirect, or not showing evidence of thalassemia, or not with complex situations like hepatopathy accompanied by iron deficiency.

Ethics Approval

This study was approved by the Medical Ethics Committee of Second Affiliated Hospital of Army Medical University, written informed consent was obtained from the patient for publication of all the data and accompanying images contained in this study. Institutional approval was required to publish the case details (institution name: the Medical Ethics Committee of Second Affiliated Hospital of Army Medical University). Institutional approval was granted to publish the case details (institution name: the Medical Ethics Committee of Second Affiliated Hospital of Army Medical University).

Consent to Participate

The patients/participants provided their written informed consent to participate in this study.

Consent for Publication

All authors/participants have seen and approved the final version of the manuscript being submitted.

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Disclosure

The authors declare no competing interests in this work.

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