

Gilbert Syndrome with Concomitant Hereditary Spherocytosis Presenting with Moderate Unconjugated Hyperbilirubinemia

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Abstract

We experienced a case of a 19-year-old man with Gilbert syndrome with concomitant hereditary spherocytosis. The patient presented with moderate unconjugated hyperbilirubinemia, and inherited etiology was strongly suspected. The diagnosis of Gilbert syndrome was confirmed by the genetic analysis of the *UGT1A1* gene, demonstrating *UGT1A1**28 and compound heterozygote *UGT1A1**6. In addition, since the laboratory findings and imaging studies revealed lysemia as well as gallstone and splenomegaly, a diagnosis of hereditary spherocytosis was made as a comorbidity.

Both Gilbert syndrome and hereditary spherocytosis are hereditary diseases with a high frequency, and the hyperbilirubinemia may be exacerbated when these two diseases are concomitant.

Key words: Gilbert syndrome, UGT1A1, hereditary spherocytosis, unconjugated hyperbilirubinemia

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Introduction

Gilbert syndrome is a genetic liver disorder producing elevated unconjugated bilirubin, often incidentally found in healthy people and patients with unrelated disease (1). Gilbert syndrome is the most common inherited metabolic liver disorder, occurring in 5-6% of the population. The elevation of serum bilirubin is usually mild, less than 6 mg/dL. Most cases of Gilbert syndrome are noticed due to slight jaundice after pubescence or found as mild elevation of unconjugated bilirubin by chance during a blood examination. Gilbert syndrome is caused by a genetic mutation of the bilirubin UDP-glucuronosyltransferase gene (*UGT1A1*), resulting in a decreased activity of UGT1A1 and an impairment of glucronidation of unconjugated bilirubin within hepatocytes (2).

In contrast, hereditary spherocytosis is the most common congenital hemolytic anemia in Japan (3). The etiology of

hereditary spherocytosis is abnormalities of the erythrocyte membrane protein. In hereditary spherocytosis, erythrocytes with a defective membrane protein lose the ability to change shape and turn into small spherocytes, which are destructed in the spleen, leading to hemolytic anemia and unconjugated bilirubinemia (3). We herein report a case of Gilbert syndrome and hereditary spherocytosis, both of which developed at 19 years of age. Most cases of Gilbert syndrome and hereditary spherocytosis develop in childhood, and therefore our case with adult onset is remarkable.

Case Report

A 19-year-old man presented with abdominal pain, nausea, fever and jaundice and visited a local clinic. A physical examination revealed jaundice, and elevation of unconjugated hyperbilirubinemia (total bilirubin 8.1 mg/dL and direct bilirubin 0.8 mg/dL) was observed in a blood chemistry

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Table. Laboratory Data at Presentation.

Complete blood count		Blood chemistries		Urinary findings	
white blood cell	7,400 / μ L (normal range, 3,000-9,800 / μ L)	total protein	6.9 g/dL (normal range, 6.5-8.2)	specific gravity	1.022
red blood cell	427×10^4 / μ L (normal range, $396-585 \times 10^4$)	albumin	4.5 g/dL (normal range, 3.7-5.5)	protein	negative
hemoglobin	13.8 g/dL (normal range, 12.5-17.6)	total bilirubin	6.66 mg/dL (normal range, 0.1-1.2)	sugar	negative
hematocrit	37.6 % (normal range, 36.8-50.5)	direct bilirubin	0.05 mg/dL (normal range, 0.10-0.40)	occult blood	negative
platelet counts	16.9×10^4 / μ L (normal range, $15-38 \times 10^4$)	aspartate aminotransferase(AST)	16 U/L (normal range, 11-32)	urobilinogen	2+
reticulocytes	3.0 % (normal range, 0.6-2.0)	alanine aminotransferase(ALT)	15 U/L (normal range, 6-35)	bilirubin	negative
reticulocytes (count)	12.8×10^4 / μ L (normal range, $2.4-8.4 \times 10^4$)	lactate dehydrogenase(LDH)	193 U/L (normal range, 119-229)		
		alkaline phosphatase(ALP)	163 U/L (normal range, 115-359)		
		γ -glutamyl transpeptidase(GGT)	33 U/L (normal range, 11-67)		
		cholinesterase	293.0 U/L (normal range, 242-495)		
direct Coombs test	negative	haptoglobin	4 mg/dL (normal range, 19-170)		

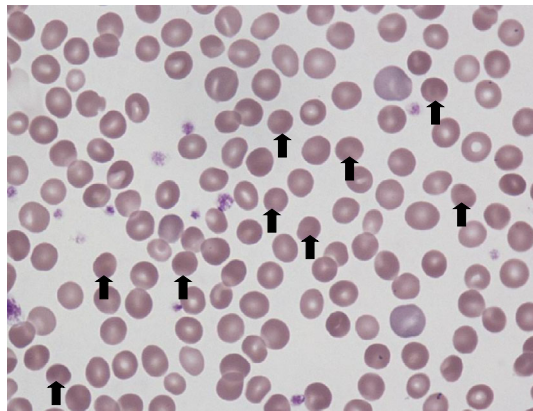


Figure 1. Peripheral blood smear ($\times 1,000$). Under normal conditions, red blood cells are flat in shape, and the centers are dented and look pale. In this patient, however, the centers of some red blood cells were deep and globular (arrow).

analysis. Elevations of the liver enzymes were not found. Both his mother and aunt also had a history of jaundice, and his aunt had been treated with phenobarbital.

The patient was introduced to Teikyo University Hospital for further examinations. His laboratory data are shown in Table, demonstrating moderate unconjugated hyperbilirubinemia. No liver injury was found. Given the unconjugated hyperbilirubinemia and presence of a family history, Crigler-Najjar syndrome type II was suspected at this time. His reticulocytes were increased both in ratio (3.0%) and number

(128,000/ μ L), even though anemia was not observed. Urobilinogen was strongly positive in his urine. His haptoglobin levels were extremely low, and the direct Coombs test was negative. A peripheral blood smear revealed red blood cell size disparity and the presence of spherocytes (Fig. 1). Abdominal computed tomography (CT) revealed splenomegaly and calcified gallstones (Fig. 2). Given these findings, we suspected hereditary spherocytosis as a comorbidity in this patient.

We then conducted a genetic analysis of *UGT1A1* of this patient for a definitive diagnosis. The genetic diagnosis for this patient was approved by the Institutional Review Board and performed with the written informed consent from the patient. The analysis revealed a T-3279G heterozygote of phenobarbital-responsive enhancer module (gtPBREM) in the enhancer region, A(TA)⁷TAA heterozygote (wild type: A(TA)⁶TAA) in the promoter region (*UGT1A1**28), and c.211G>A:p.G71R (*UGT1A1**6) heterozygote. Since T-3279G and A(TA)⁷TAA are generally linked, the mutation of this case was diagnosed as T-3279G +A(TA)⁷TAA with a compound heterozygote of G71R. Taken together with the previous findings, the genetic analysis confirmed the presence of Gilbert syndrome in this patient, concomitant with hereditary spherocytosis (4, 5).

Discussion

Bilirubin, produced from catabolization of heme, was

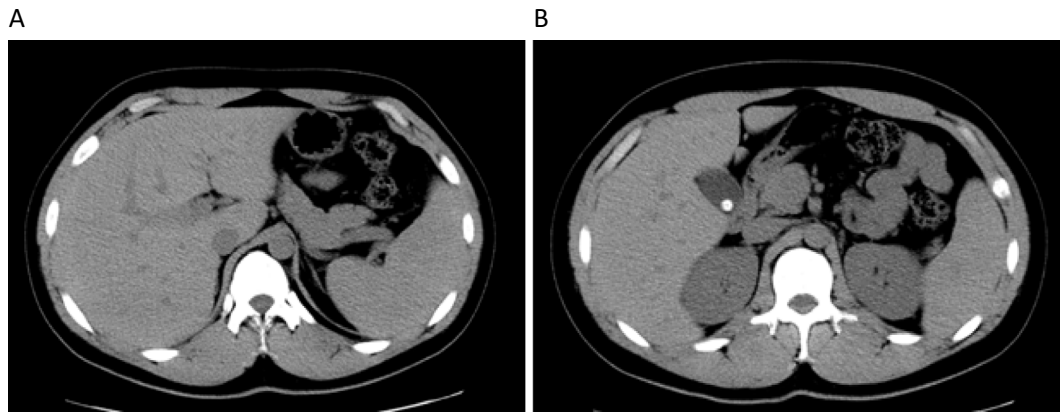


Figure 2. Abdominal CT. Splenomegaly (A) and calcified gallstones (B) were observed.

transported into hepatocytes and conjugated with glucuronic acid in a process called glucuronization by the UGT1A1 enzyme. The reduced enzymatic activity of UGT1A1 due to the somatic mutation of the *UGT1A1* gene located on chromosome 2 results in unconjugated hyperbilirubinemia, which is known as Crigler-Najjar syndrome and Gilbert syndrome. Crigler-Najjar syndrome is an inherited metabolic liver disorder with two types: type I and type II. The enzymatic activity of UGT1A1 is completely lacking in type I and reduced to 10% or less in type II. As a result, Crigler-Najjar syndrome is diagnosed by profound neonatal unconjugated hyperbilirubinemia. In Gilbert syndrome, on the other hand, the activity of UGT1A1 is reduced to 30% or less, and therefore hyperbilirubinemia is usually mild in Gilbert syndrome.

To make a definitive diagnosis of Gilbert syndrome, a genetic analysis of *UGT1A1* gene is required. In general, T-3279G in the enhancer gPBREM, A(TA)⁷TAA in the promoter region, and the missense mutation p.G71R of exon 1 are usually reported as genetic mutations contributing to Gilbert syndrome (2, 4, 5). In most cases, A(TA)⁷TAA is linked with T-3279G, forming the allele T-3279G + A(TA)⁷TAA (*UGT1A1**28). The frequencies of A(TA)⁷TAA and T-3279G are 0.15 and 0.16 in Japanese, respectively, explaining the high frequency of Gilbert syndrome. Hyperbilirubinemia in Gilbert syndrome depends on the status of these variations-homozygote or compound heterozygote. In addition, p.G71R (*UGT1A1**6) is a characteristic polymorphism exclusive to East Asia and has never been reported in other races. Furthermore, p.Y486D(*UGT1A1**7) in exon 5, and T-3279G + A(TA)⁷TAA + p.P229Q(*UGT1A1**27), p.L364P(*UGT1A1**63), p.R209W(*UGT1A1**8), and p.R367G(*UGT1A1**29) have been reported in Japanese patients with Gilbert syndrome (6). In contrast, p.C280X(*UGT1A1**25) is the most common mutation in Crigler-Najjar syndrome type I, and the duplicated missense mutation p.[G71R;Y486D] due to a point mutation of c.211G>A:p.G71R in exon 1 and a mutation of c.1456G>A:p.Y486D in exon 5 is most frequently seen in Crigler-Najjar syndrome type II in Japanese patients (6, 7). Therefore, a genetic analysis of the *UGT1A1* gene is extremely important for the differential diagnosis of

inherited unconjugated hyperbilirubinemia.

In contrast, hereditary spherocytosis is the most common congenital hemolytic anemia in Japan, accounting for about 70%, of cases. Its prevalence is presumed to be 5.7-20.3 persons per 1 million people (3). Abnormalities of erythrocyte membrane proteins, including Band 3 (SLC4A1), ankyrin (ANK1), protein 4.2 (EPB4.2), and β -spectrin (SPTB), are considered to be responsible for the pathogenesis, with abnormality frequencies of 30% for Band 3, 25% for ankyrin, and 25% for protein 4.2 in Japanese versus 60% for ankyrin and 30% for Band 3 in Caucasians. In most cases, unconjugated hyperbilirubinemia appears in newborns with hereditary spherocytosis, and the therapeutic options include phototherapy for mild jaundice and exchange transfusion for moderate to severe jaundice. Although splenectomy is also an option, such surgery is usually delayed until after immunity to avoid possible disturbance of the maturation of immunity caused by removal of the spleen (8).

In the current case, the patient developed jaundice at age 19. Given his family history of hyperbilirubinemia and the moderate elevation of bilirubin (8.1 mg/dL), we suspected Crigler-Najjar syndrome type II, not Gilbert syndrome. However, a genetic analysis revealed *UGT1A1**28 and the compound heterozygote of *UGT1A1**6, and thus a diagnosis of Gilbert syndrome was made. The serum bilirubin level was high compared to usual cases of Gilbert syndrome likely because of the coexistence of Gilbert syndrome and hereditary spherocytosis. Bilirubin levels increase when the effects of Gilbert syndrome and hereditary spherocytosis for unconjugated hyperbilirubinemia merge (9-11). Furthermore, when hereditary spherocytosis coexists with Gilbert syndrome, the risk for gallstones is increased 5-fold (12). In the current case, even though the patient was 19 years old, cholelithiasis was already observed. Therefore, cholecystectomy and splenectomy to treat the gallstones and hereditary spherocytosis, respectively, will be required in the future.

Of further note, UGT1A1 plays a role in drug metabolism, and thus drug metabolism is largely influenced by the *UGT1A1* gene polymorphisms. For instance, it has been reported that the side effects of irinotecan hydrochloride, such

as leukopenia (neutropenia) and diarrhea, are exacerbated by polymorphisms such as *UGT1A1*28* and *UGT1A1*6* (13, 14). Therefore, extreme caution should be practiced when drugs metabolized by UGT1A1 are administered in patients with Gilbert syndrome who have inherited mutations and reduced enzymatic activities.

The authors state that they have no Conflict of Interest (COI).

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