Case Report

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Hereditary Spherocytosis Coexisting with UDP-Glucuronosyltransferase Deficiency Highly Suggestive of Crigler-Najjar Syndrome Type II

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Patients with co-existing hereditary spherocytosis (HS) and UDP-glucuronosyltransferase 1A1 (UGT1A1) deficiency as Gilbert's syndrome (GS) have been reported, and previous studies have demonstrated an increased risk for developing gallstones in patients with co-inheritance of GS and HS. We experienced an interesting case of HS showing persistent jaundice after splenectomy, and upon further evaluation, the 25-year-old female patient was found to have HS combined with UGT1A1 deficiency. Sequence analysis of the UGT1A1 gene revealed that she was a compound heterozygote with p.[G71R; Y486D] + [Y486D] mutations, which suggests Crigler-Najjar syndrome type II rather than GS. Careful evaluation of inappropriately elevated bilirubin level compared with the degree of hemolysis is important, reflecting the therapeutic implication of splenectomy and cholecystectomy.

Key Words: Hereditary spherocytosis, unconjugated hyperbilirubinemia, UDP-glucuronosyltransferase, Crigler-Najjar syndrome, splenectomy

INTRODUCTION

Hereditary spherocytosis (HS) is the most common cause of hemolytic anemia due to constitutional erythrocyte membrane disorder. Most patients have well-compensated hemolysis but have icterus and cholelithiasis due to chronic hemolysis.[1] Familial non-hemolytic unconjugated hyperbilirubinemia including Crigler-Najjar syndrome type I, Crigler-Najjar syndrome type II (CN-II), and Gilbert's syndrome (GS), is associated with a defect in isoenzyme 1A1 of UDP-glucuronosyltransferase (UGT1A1).[2] Cases with co-existence of HS and UGT1A1 deficiency as GS have been reported,[3-5] and previous studies have demonstrated an increased risk for developing gallstones in patients with co-inheritance of HS and GS.[6,7] However, there are no reports on HS coexisting with other deficiency state of UGT1A1.

CASE REPORT

The patient was a 25-year-old Japanese woman who had a history of jaundice for

11 years. Her family history revealed that her father had mild jaundice while other family members had normal serum bilirubin levels. The patient received regular medical treatment at a psychiatric outpatient clinic because of truancy. She had been referred to our hospital for extensive evaluation of jaundice at the age of 14 years. Her first blood examination revealed a normal hemoglobin level (12.6 g/dL), slightly elevated reticulocyte count (89 × 10^6 /L: reference range, 40 to 80 × 10^6 /L), and unconjugated hyperbilirubinemia (serum total bilirubin 113 μmol/L) without elevation of liver-associated enzymes. A diagnosis of HS was made based on clinical features including chemical (haptoglobin < 1.0 μmol/L) and hematologic findings (spherocytes on blood smear and increased red cell osmotic fragility). However, biochemical analysis of the red cell membrane proteins indicated no significant deficiency. Thereafter, she persistently demonstrated mild jaundice but not anemia. At the age of 16 years, she underwent splenectomy to prevent the formation of gallstones and to reduce skin itching which was probably induced by jaundice. Subsequently, the reticulocyte count decreased and the serum haptoglobin level normalized. However, she was persistently positive for serum bilirubin, which ranged between 86 and 103 μmol/L, and it was not clear whether her serum bilirubin level improved after splenectomy. Therefore, we suspected somatic hyperbilirubinemia and carried out further examinations. Physical examination was remarkable only for icteric sclera. Extensive laboratory evaluation revealed normal levels of liver-associated enzymes and lactate dehydrogenase (LDH). The haptoglobin level was 6.0 μmol/L with a total bilirubin of 102 and unconjugated fraction of 100 μmol/L. A complete blood count revealed normal numbers of white blood cells and platelets with a hemoglobin level of 12.4 g/dL. The corrected reticulocyte count was 34 × 10^6 /L. A peripheral blood smear revealed spherocytes. Incubated osmotic fragility was slightly increased. Sequence analysis of the UGT1A1 gene revealed that the patient was heterozygous for a missense mutation (c.211G > A: p.G71R in exon 1) and homozygous for a point mutation (c.1456T > G: p.Y486D in exon 5); that is, she was a compound heterozygote with the p.[G71R; Y486D] + [Y486D] mutations (Fig. 1). As for the parents of the patient, detailed laboratory data could not be obtained, and neither a molecular study of their UGT1A1 gene nor analysis of their erythrocyte membrane proteins was performed.

DISCUSSION

According to the diagnostic criteria of HS,[1] patients with no anemia and mild reticulocytosis are classified as having the mild type (hemoglobin level from 11 to 15 g/dL, reticulo-

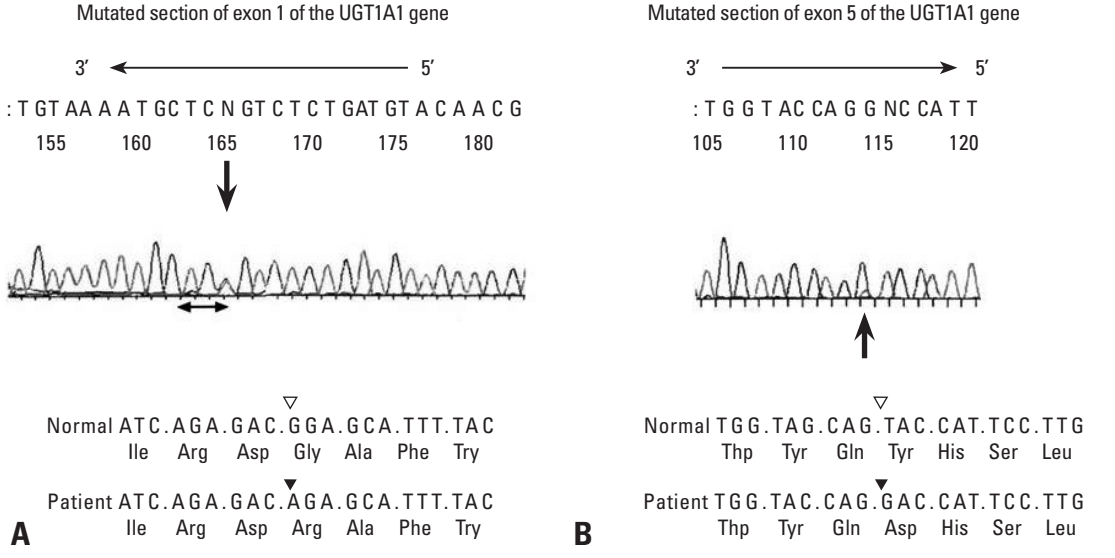

Fig. 1. Nucleotide sequences of the mutated sections of exons 1 and 5 and the promoter region of the gene encoding UDP-glucuronosyltransferase 1A1 (UGT1A1). (A) Nucleotide sequences of the mutated section of exon 1 of the UGT1A1 gene. A missense mutation at nucleotide 211 in exon 1 of the UGT1A1 gene is observed. The substitution of adenine for guanine changed the codon from GGA to AGA, causing arginine to replace glycine at position 71 (p.G71R in the corresponding protein). An arrow shows the mutation. The patient was heterozygous for p.G71R. (B) Nucleotide sequence of the mutated section of exon 5 of the UGT1A1 gene. The arrow shows the mutation. The patient was homozygous for a point mutation at base position 1456 in exon 5. The substitution of guanine for thymine changed the codon from TAC to GAC, causing a tyrosine-to-aspartic acid substitution at position 486 (p.Y486D in the corresponding protein).

cyte count from 3% to 6%, total bilirubin level from 17 to 34 mg/dL). In contrast, patients with higher bilirubin levels are classified as having either the moderate type (total bilirubin level from 34 to 51 mg/dL) or the severe type (more than 51 mg/dL). Although our patient had mild HS with normal hemoglobin and slightly elevated reticulocyte count, she had an extremely high bilirubin level. This discrepancy suggested other conditions associated with unconjugated hyperbilirubinemia, especially inherited deficiency of hepatic glucuronosyltransferase. In such cases, further evaluation should be performed. Similar cases of coexisting HS and GS have been reported.[4,8] Since the prevalence of GS in the general population is 5-7%[9] and that of HS is one per 2000, the calculated rate of co-occurrence of these two disorders is approximately 25-35 per one million births. In contrast, the incidence of CN-II is one per one million births.[10] Therefore, the calculated rate of co-occurrence of HS and CN-II indicates that it is extremely rare.

Similar to GS, the method of choice for diagnosis of Crigler-Najjar syndrome is mutation analysis of genomic DNA. The phenotype of CN-II is usually associated with homozygosity or compound heterozygosity for missense mutations in the UGT1A1 gene that do not completely inactivate the protein.[11,12] Our patient was a compound heterozygote with the p.[G71R; Y486D] allele and p.Y486D allele. However, most Japanese cases with CN-II have been caused by a homozygous double missense mutation (p.[G71R; Y486D] + [G71R; Y486D]),[13,14] and the mutation found in our case has not been reported as a cause of CN-II.[2,15] According to a previous expression study, the relative UGT1A1 activity of a single homozygous model of p.G71R was approximately 30% of normal, that of a single homozygous model of p.Y486D was 7% to 8%, and that of a double homozygous model of p.[G71R; Y486D] + [G71R; Y486D] was 4% to 7%.[14] It has been reported that hepatic UGT1A1 activity (measured from liver tissue) in patients with CN-II is less than 10% of normal, whereas that in patients with GS is about 30% of normal.[16,17] We concluded that the persistent hyperbilirubinemia after splenectomy in our patient was due to CN-II rather than GS, based on findings from sequence analysis of the UGT1A1 gene.

When cholelithiasis is present, prophylactic cholecystectomy is performed at the same surgical setting as splenectomy in patients with mild HS.[18] However, combined prophylactic splenectomy and cholecystectomy are not recommended for patients with HS with no evidence of cholelithiasis, because it is thought that individuals with HS do not develop pigment stones once the spleen is removed.[19] A patient with HS who coinherited GS, has an almost 5-fold greater tendency to form gallstones than a normal HS patient.[6] Coexistence with CN-II might further increase the risk of gallstone formation. Therefore, in cases of HS combined with reduced hepatic bilirubin conjugation, it is unknown whether splenectomy alone can sufficiently reduce hemolysis and prevent subsequent gallstone formation. Combined prophylactic splenectomy and cholecystectomy might be recommended for cases like ours. Careful evaluation of inappropriately high serum bilirubin level compared with the degree of hemolysis as defined in the diagnostic criteria of HS[1] is important, reflecting the therapeutic implication of splenectomy and cholecystectomy.

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