Clinical Practice

Mixed Dubin-Gilbert Syndrome: A Compound Heterozygous Phenotype of Two Novel Variants in ABCC2 Gene

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INTRODUCTION

Gilbert syndrome (GS, MIM #143500) is characterized by fluctuating mild, unconjugated hyperbilirubinemia (<85 μmol/L) and is caused by mutations in the bilirubin uridine diphosphate (UDP)‑glucuronosyltransferase gene (UGT1A1).[1] Dubin-Johnson syndrome (DJS, MIM #237500) is characterized by fluctuating mild, predominantly conjugated hyperbilirubinemia and is caused by mutations in the ATP‑binding cassette subfamily C member 2 gene (ABCC2).[2] This report described the unusual features encountered in a Chinese family that was genetically confirmed to have both DJS and GS.

CASE REPORT

A 31-year-old woman presented with a consistent, mild elevation of serum bilirubin lasting about 28 years. Physical examination after admission to our hospital showed that she was febrile, with normal blood pressure and heart rate, and of height 160 cm and weight 50 kg. She had no skin rash, lymphadenopathy, or hepatosplenomegaly, and no tenderness on palpation of the abdomen. Her skin and sclera, however, were noticeably yellow. Her family history was negative. Tests performed to determine whether she had jaundice showed increased concentrations of total serum bilirubin (T‑Bil 103.9 μmol/L) and direct bilirubin (D‑Bil 41.8 μmol/L), along with fever, vomiting, and scleral icterus. She was serologically negative for hepatitis A, B, C, and E viruses; Epstein-Barr virus; and cytomegalovirus. Her liver and bile duct system were normal on ultrasonography, computed tomography, and magnetic resonance imaging. ⁹⁹ᵐTe-HIDA cholescintigraphy revealed prolonged visualization of the liver and delayed filling of the gallbladder. Her serum concentrations of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, α-antitrypsin, copper, ceruloplasmin, and thyroid hormones were all within normal ranges. Her sweat chloride concentration and screening biochemistries for metabolic diseases were also within normal ranges, and she was negative for tumor-associated antigens and autoantibodies.

Microscopic examination of the liver biopsy tissue showed deposits of black pigment in liver cells. This pigment displayed staining properties of lipofuscin and melanin, with strong Schmorl positivity as well as strong autofluorescence [Figure 1a]. Accumulation of melanin-like pigment represented a characteristic histomorphologic feature of DJS [Figure 1b].

The study protocol was approved without restrictions by the Medical Ethics Committee of the China-Japan Friendship Hospital, and informed consent was obtained from the patient and her parents. Blood (2 ml) was drawn for Sanger sequencing from the patient, her healthy parents, and 100 unrelated normal controls. Sequencing of polymerase chain reaction‑amplified DNA of the entire coding regions and exon‑intron boundaries of UGT1A1 (NM_000463.2) and ABCC2 (NM_000392.3) in the patient revealed two novel mutations [Figure 1c]: a heterozygous in-frame insertion

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deletion mutation c.4712_4720delinsGGCCCACAG in exon 31, inherited from her father, and a heterozygous mutation p.I448V in exon 10, inherited from the mother. Neither of these mutations had been observed in the dbSNP and 1000 genomes databases indicating that these variants are very rare. In addition, the patient appeared to be homozygous for the A(TA)7TAA allele and the T>3279G mutation [Figure 1c] in the UGT1A1 promoter.

To further test if these two novel mutations are indeed potential pathogenic factors, the ABCC2 sequence variations resulting from c.4712_4720delinsGGCCCACAG and p.1448V were both tested by MutationTaster, with both found to be disease-causing mutation. Based on PolyPhen-2 (Harvard Medical School, Boston, Massachusetts, USA), the 1448V mutation was found to be “probably damaging” to protein structure and function, with a score of 0.999. In addition, the I-Mutant 2.0 server, which predicts the change in free energy (sign of DDG), found that the new p.1448V mutation decreased the stability of the protein [Supplementary Table 1].

**DISCUSSION**

Our evidence suggests that the linkage of T>3279G with A(TA)7TAA is essential for the pathogenesis of GS. The combined homozygosity of these two genetic alterations in the UGT1A1 promoter can reduce the transcriptional activity of this gene to <30% of normal.[1] In addition, higher bilirubin levels were found in patients with the p.1448V mutation compared to those with no mutations, suggesting that this mutation may severely disrupt transporter activity.[6] The combination of this mutation with the frame shift mutation in a compound heterozygote may play a role in the slow clearance of conjugated bilirubin and/or the expression of enzymes involved in the cyclization of hydroxymethylbilane. The complete loss of ABCC2 function resulting from compound heterozygous mutations may therefore result in DJS.

The simultaneous presence of mutations characteristic of DJS and GS has been classified as dual hereditary jaundice.[5] Our findings demonstrated the occurrence of both GS and DJS phenotypes in a Chinese patient with pathogenic mutations in the ABCC2 gene and the UGT1A1 promoter. These findings suggest methods for a more efficient diagnosis of this dual disorder in Chinese patients as well as genetic counseling of carriers. Additional studies, however, are required to determine the biological mechanism by which these mutations affect bilirubin transporter activity.

*Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.*

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Conflicts of interest
There are no conflicts of interest.

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## Supplementary Table 1: Bioinformatics analysis of two novel pathogenic mutations

| Novel mutation | PolyPhen-2* | Bioinformatics analysis | I-mutant 2.0 (sign of DDG)† | Domain type |
|----------------|-------------|-------------------------|-----------------------------|-------------|
|                | Prediction  | Score       | Prediction       | Score       | Prediction       |
| p.I448V        | Probably damaging | 0.999 | Disease causing | 0.999 | Decrease stability | Transmembrane |
| c.4712_4720delinsGGCCACAG | – | – | Disease causing | 0.911 | – | AAA+ ATPase domain |

*PolyPhen-2 Prediction Score: Benign ≤0.5; probably damaging (>0.5); †I-Mutant 2.0 prediction: Sign of DDG: Decrease stability or increase stability; ‡MutationTaster prediction: Polymorphism or disease causing.