CASE REPORT

Dual Hereditary Jaundice: Simultaneous Occurrence of Mutations Causing Gilbert's and Dubin–Johnson Syndrome

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Background & Aims: **Dubin–Johnson syndrome is recessively inherited, conjugated hyperbilirubinemia induced by mutations in the *ABCC2/MRP2* gene encoding the canalicular transporter for conjugated bilirubin. Gilbert's syndrome is recessively inherited, unconjugated hyperbilirubinemia caused by decreased conjugation rate of bilirubin associated mostly with homozygous $A(TA)_7TAA$ variant of the TATAA-box in the *UGT1A1* gene promoter. Our aim was to establish the molecular diagnosis in a 3-year-old male with atypical, intermittent, predominantly unconjugated, hyperbilirubinemia.** ***Methods:*** **^{99m}Tc-HIDA cholescintigraphy was used for imaging the biliary tree. Expression of ABCC2/MRP2 protein in hepatocytes was investigated immunohistochemically. *UGT1A1* and *ABCC2/MRP2* genes were sequenced from genomic DNA, and the mutations were verified by fragment analysis, sequencing the cloned exons, and restriction fragment length polymorphism.** ***Results:*** **Cholescintigraphy revealed delayed visualization of the gallbladder. A brown granular lipopigment differing from melanin-like pigment reported in Dubin–Johnson syndrome was present in hepatocytes, but, otherwise, liver histology was normal. ABCC2/MRP2 protein was not detected on the canalicular membrane of hepatocytes, and 2 novel mutations were found in the *ABCC2/MRP2* gene: a heterozygous in-frame insertion-deletion mutation 1256insCT/delAAACAGTGAACCTGATG in exon 10 inherited from the father and a heterozygous deletion 4292delCA in exon 30 inherited from the mother. In addition, the patient was homozygous for −3279T>G and $A(TA)_7TAA$ mutations in the *UGT1A1* gene promoter.** ***Conclusions:*** **Our patient represents a case of digenic mixed hyperbilirubinemia—a distinct type of constitutive jaundice resulting from coinherited defects in *ABCC2/MRP2* and *UGT1A1* genes.**

The excretory pathway for bilirubin consists of 2 sequential steps: glucuronidation of unconjugated bilirubin catalyzed by UDP-glucuronosyl transferase 1A1 (UGT1A1) and biliary secretion of conjugated bilirubin via ABCC2/MRP2, the bilirubin export pump expressed on the canalicular membrane of hepatocytes. Impairment of bilirubin glucuronidation because of reduced activity of UGT1A1 causes Gilbert's syndrome (GS; On-line Mendelian Inheritance in Man database [OMIM] No. 143500), the most frequent hereditary hyperbilirubinemia affecting 5%–10% of the white population.[1,2] The disorder is characterised by mild, chronic, fluctuating, unconjugated hyperbilirubinemia with serum bilirubin levels up to 6.0 mg/dL. Mutations in the *UGT1A1* gene are responsible for the molecular basis of GS.[3,4] An insertion of 1 TA repeat in the TATAA box of the *UGT1A1* gene promoter, named also *UGT1A1*28* allele, is the almost exclusive mutation present in the homozygous state in 11%–16% of European and North American populations.[4,5]

Dubin–Johnson syndrome (DJS; OMIM No. 237500) is a rare, benign, predominantly conjugated hyperbilirubinemia with typical deposits of melanin-like pigment within hepatocyte lysosomes. ^{99m}Tc-HIDA cholescintigraphy is characterized by prolonged homogenous visualization of the liver with delayed filling of the gallbladder.[6,7] Total urinary porphyrin output is normal, but the ratio of urinary coproporphyrin isomers I and III is shifted from 1:3 to 4:1. DJS has an autosomal recessive mode of transmission, and mutations in the *ABCC2/MRP2* gene are responsible for the phenotype (Table 1).

Abbreviations used in this paper: ABCC2, ATP-binding cassette protein C2; BSP, bromosulphthalein; CEACAM1, CEA-related adhesion molecule 1; DAPI, 4',6-diamidino-2-phenylindole; DJS, Dubin–Johnson syndrome; GS, Gilbert's syndrome; HIDA, 2,6-dimethylacetanilido-iminodiacetic acid; MRP2, multidrug resistance related protein 2; PAS, periodic acid Schiff; PCR, polymerase chain reaction; RFLP, restriction fragment-length polymorphism; UGT1A1, UDP-glucuronosyl transferase 1A1.

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Presumably because of the frequency of GS, a significant number of the individuals affected with DJS present with predominantly unconjugated hyperbilirubinemia. Combination of DJS with GS was suggested as an explanation for such atypical variant of DJS in the case reported by Tanikawa and Abe.[8] In our report, we present the first case of this digenic hyperbilirubinemia proven at the molecular level.

Materials and Methods

Case Report

Our patient is a 3-year-old boy born after a full-term and uneventful pregnancy as the first child of unrelated parents. The family history was negative. Neonatal jaundice was managed by phototherapy for 5 days. The boy was breast-fed for 3 months, thrived well, and had no jaundice. At the age of 2 years, the patient was examined for allergic exanthema after administration of amoxicillin/clavulanate (potassium salt), and mild hyperbilirubinemia was detected (total bilirubin 2.1 mg/dL, direct bilirubin 0.5 mg/dL). Serologic screening tests for hepatotropic viruses (hepatitis A, B, and C; Epstein–Barr virus; and cytomegalovirus) were negative. Other laboratory findings were within the normal range. Bile duct obstruction was excluded by ultrasonography, and serum bilirubin level dropped spontaneously into the normal range. One year later, fever, vomiting, and scleral icterus appeared; increased serum levels of both total (5.6 mg/dL) and direct reacting (2.3 mg/dL) bilirubin were detected; and the boy was admitted to the hospital. Complete blood count was within the normal range, total serum bilirubin level decreased to 3.0 mg/dL and direct reacting fraction to 0.7 mg/dL. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase, α1-antitrypsin, copper, ceruloplasmin and thyroid hormones, sweat chloride concentration, and screening biochemistries for metabolic diseases were all within the normal range. Urinary coproporphyrin output was normal (40 μg/24 hours), but the level of isomer I was increased to 94%. There was no evidence of infection with hepatotropic viruses. No pathology was observed on ultrasonography of the liver and bile ducts. ^{99m}Tc-HIDA cholescintigraphy revealed prolonged visualization of the liver and delayed filling of gallbladder. Laboratory examination of the parents, based on their informed consent, revealed mild, unconjugated hyperbilirubinemia in the mother (total bilirubin, 1.7 mg/dL).

Histology and Immunohistochemistry

Histology. Liver biopsy specimens were stained with H&E, periodic acid Schiff (PAS) method, and silver ammonium complex (Masson's method).

Antibodies. The anti-MRP2 mouse monoclonal antibody (clone M_2III-6) was purchased from Kamiya (Seattle, WA). The rabbit polyclonal anti-human carcinoembryonic antigen (CEA) antibody, which recognizes CEA-related adhesion molecule 1 (CEACAM1) on biliary canaliculi of human liver, was purchased from DAKO (Glostrup, Denmark) together with the EnVision Peroxidase Kit and LSAB+ Kit. Fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit antibody and Cy5-conjugated goat anti-mouse antibody were obtained from Jackson (West Grove, PA).

Immunohistochemistry. For immunohistochemical procedures, 5-μm-thick sections cut from formalin-fixed, paraffin-embedded tissue samples were deparaffinized and pretreated with 2.7% hydrogen peroxide and 0.1% sodium azide. The slides were incubated with primary mouse anti-MRP2 monoclonal antibody diluted to 1:20 with Tris-buffered saline (TBS) containing 5% fetal calf serum and primary rabbit anti-CEA polyclonal antibody, diluted to 1:1000 in the same buffer. The EnVision Peroxidase Kit and the LSAB+ Kit were used to visualize sections incubated with primary mouse monoclonal antibody and primary rabbit antibody, respectively. The chromogen 3,3-diaminobenzidine from FLUKA (Buchs, Switzerland) was applied to all sections, and counterstaining was performed with Harris's hematoxylin. As a positive control, sections of an adult liver with minimal hepatopathy without cholestasis were stained. Liver sections incubated without primary antibodies were used as negative controls.

Table 1. Mutations in the *ABCC2/MRP2* Gene Associated With Dubin–Johnson Syndrome

Mutation	Exon	Consequence	Reported by
298C>T	3	R100Stop	Shoda J et al, Hepatol Res 2003;27:323–326.
1031 + 4A>G	8	Complex splicing	Mor-Cohen R et al, Hepatol Res 2005;31:104–111.
1815 + 2T>A	13	Skipped exon	Wada M et al, Hum Mol Genet 1998;7:203–207.
1967 + 2T>C	15	Skipped exon	Kajihara S et al, Biochem Biophys Res Commun 1998;253:454–457.
2026G>C	16	G676R	Wakusawa S et al, J Hum Genet 2003;48:425–429.
2125T>C	17	W709R	Machida I et al, Hepatol Res 2004;30:86–90.
2302C>T	18	R768W	Wada M et al, Hum Mol Genet 1998;7:203–207.
2439 + 2T>C	18	Skipped exon	Wada M et al, Hum Mol Genet 1998;7:203–207.
3196C>T	23	R1066Stop	Paulusma CC et al, Hepatology 1997;25:1539–1542.
3449G>A	25	R1150H	Mor-Cohen R et al, J Biol Chem 2001;276:36923–36930.
3517A>T	25	I1173F	Mor-Cohen R et al, J Biol Chem 2001;276:36923–36930.
3928C>T	28	R1310Stop	Tate G et al, Genes Genet Syst 2002;77:117–121.
4145A>G	29	Q1382R	Toh S et al, Am J Hum Genet 1999;64:739–746.
4175delGGATGA	30	Del RM	Tsujii H et al, Gastroenterology 1999;117:653–660.