SNP Communication

Novel Large-scale Deletion (whole Exon 7) in the ABCC2 Gene in a Patient with the Dubin-Johnson Syndrome

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Summary: The Dubin-Johnson syndrome (DJS) is an inherited liver disorder characterized by conjugated hyperbilirubinemia and caused by ABCC2 gene mutations resulting in deficiency of multidrug resistance associated-protein 2 (MRP2) function. A 76-year-old woman with serious jaundice was referred to our hospital. She was clinically diagnosed with DJS with hepatic congestion, due to constrictive pericarditis. We analyzed all exons and exon-intron junctions of the ABCC2 gene by DNA sequencing and identified a new large-scale deletion, 1008 bp, including the whole exon 7, as homozygosity. Some mutations in the ABCC2 gene associated with splicing errors have been reported in intronic regions; however, this is a new type of large-scale deletion detectable in the genomic DNA sequence. Severe hyperbilirubinemia is rare in patients with constrictive pericarditis and this case suggests that MRP2 may play a crucial role in compensating for the serum bilirubin in congestive hepatopathy.

Keywords: MRP2; ABCC2; Dubin-Johnson syndrome; large-scale deletion; single nucleotide polymorphism

Introduction

The Dubin-Johnson syndrome (DJS) is an autosomal recessive disorder characterized by a defect in the biliary excretion of bilirubin glucuronides, typical endogenous substrates for multidrug resistance associated-protein 2 (MRP2), leading to chronic conjugated hyperbilirubinemia.1) DJS is caused by deficient transport capability of MRP2 (gene ABCC2). In the liver, MRP2 mediates the multispecific efflux of various types of organic anions, including glucuronate, sulphate and glutathione conjugates across the canalicular hepatocyte membrane into the bile. The human ABCC2 gene, located at chromosome 10q24, spans about 45 kb and contains 32 exons, ranging in size from 56 to 255 bp. Various mutations have been identified in DJS patients.2–5) The most frequent mutations are single mutations, but their consequences at the cDNA level are multiple, creating stop codons and skipping exons. Genetic analysis of DJS is then generally used for both genomic DNA and cDNA to analyze samples. Recently, polymorphism of the ABCC2 gene has been suggested to play important roles in large inter-individual variabilities in pharmacokinetic/pharmacodynamic (PK/PD) profiles of certain clinically relevant drugs. For

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example, remarkable reduction in the methotrexate elimination rate (with three-fold reduction) was observed after high-dose infusion in a patient with DJS with loss-of-function mutations in the ABCC2 gene.6)

**Materials and Methods**

**Case presentation:** The patient was a 76-year-old Japanese woman clinically diagnosed with DJS. She had been well other than jaundice and black liver noted when she underwent cholecystectomy for gallstones at the age of 70. The serum total bilirubin had been elevated approximately to 4 mg/dL. Pleural effusion was treated by her local medical clinic and pericardial thickening was noted on the chest computed tomography scan. In her family history, consanguineous marriage had occurred for four generations; however, she was the only family member with constitutional jaundice. She was referred to our hospital because of worsening jaundice and slight pleural effusion in June, 2002. Despite worsening jaundice, no significant symptoms could be observed. Her jugular vein was dilated but her liver and spleen were not palpable. There was no peripheral edema. The serum total bilirubin level was over 40 mg/dL, most of which was conjugated. Otherwise, laboratory findings, such as alanin and aspartate aminotransferases, alkaline phosphatase, gamma-glutamyl transpeptidase, and prothrombin time, were within the normal range. Abdominal ultrasonography revealed conspicuous enlarged hepatic veins, suggesting significant hepatic congestion. Cardiomegaly was not shown on the chest X-ray. Dip and plateau patterns and extreme increase in the right atrial pressure were noted in the cardiac catheterization study. Histopathologic examination of a liver biopsy sample revealed brown pigment accumulation in hepatocytes, which is characteristic of DJS (Fig. 1a). Many bile thrombi were seen in the canaliculi but fibrosis did not involve terminal hepatic venules and the portal area. Thus, she was diagnosed as hepatic congestion due to constrictive pericarditis with DJS. Partial pericardectomy was performed and jaundice markedly improved, depending on decrease in right atrial pressure (Fig. 1b).

**PCR and sequence analysis:** Genomic DNA was isolated from a blood sample using the Toyobo blood kit on a Toyobo HMX-200 robot (Toyobo, Osaka, Japan). The primer design was based on published sequences.5,7) Polymerase chain reaction (PCR) was carried out to amplify all 32 exons, exon-intron junctions and the 5′-untranslated flanking region of the ABCC2 gene. The designed primer sets and PCR conditions have been reported elsewhere.8) All PCR products were sequenced on an ABI 3100 automatic sequencer using a Big-Dye Terminator Cycle Sequencing kit (Applied Biosystems). The primers used to amplify the deletion region, including exon 7, were as follows: prA, 5′-ATCTTGCAGTAA-

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**Fig. 1. Tissue staining (a) and time courses of total bilirubin and right atria pressure (b)**
(a) (1) Fibrosis could hardly be seen with Azan staining (magnification ×200). (2) Histological specimen with H&E staining (original magnification ×400) shows brown granular deposits (arrows) in hepatocyte cytoplasm. (b) Partial pericardectomy significantly decreased right atrial pressure (RAP; dotted line). Consequently, jaundice markedly improved (solid line). Arrow shows date of operation.
Fig. 2. Sequence analysis of the ABCC2 gene around exon 7 amplified from genomic DNA of the patient using primers prA and prB. Sequence at intron 6/7 boundary was indicated (reverse primers were used); deletion of exon 7 was clearly observed. A 1008 bp deletion was observed in the patient (from IVS 6-275 to IVS 7+498), including the whole of exon 7 (Fig. 2). Based on the predicted topology of MRP2, exon 7 has been shown located in the intra-cellular loop between membrane-spanning domain 1 and 2. This case was clinically diagnosed as DJS and the patient subsequently suffered from marked hyperbilirubinemia because of hepatic congestion. This is the first report of a DJS patient with very severe jaundice due to hepatic congestion resulting from constrictive pericarditis and marked improvement by partial pericardectomy. We identified a novel mutation, a 1008 bp deletion including the whole of exon 7, in the ABCC2 gene.
significant variation in the on its localization in human tissue, functionally sig-

ABCC2 methotrexate,6) irinotecan,13) and pravastatin.14) The un-

bilirubin. It remains unclear why hepatic congestion in-

Serum bilirubin commonly occurs in up to 70% of these 

patients.10) Contributing factors may include liver cell 

dysfunction, hemolysis, pulmonary infarction and 
canalicular obstruction.9) No factors were noted in this 

case. Constrictive pericarditis causes higher hepatic vein 

pressure than seen in right-sided heart failure and hepatic 
necrosis and ultimately cirrhosis. Hyperbilirubinemia is 

rare in patients with constrictive pericarditis diagnosed 

before developing liver fibrosis.11) This histological fea-

ture revealed hepatic necrosis but hardly any severe 

fibrosis or cirrhosis. This study suggests that MRP2 pro-

tein may profoundly participate in compensating for 

hyperbilirubinemia caused by congestive hepatopathy; 

however, its mechanism could not be clarified in this 

case.

While screening the ABCC2 gene for mutations, we 

identified two SNPs in this patient: one was in the non-
coding region, \( −24C>T \), and the other in the coding 

region of the gene, \( 3972C>T \) (synonymous SNP), in 
exon 28. These SNPs can be seen at high frequency, 

18.8% and 21.9%, respectively, in Japanese.8) Interest-

ingly, mutation \( 3972C>T \) was highly linked with 

\( −24C>T \) (i.e., haplotype formation) (Ref. 12 and our 

unpublished data in Japanese). Although the \( −24C>T \) 

variant has not been reported associated with DJS, a 

recent study indicates that this variant is associated with 

changes in the PK/PD properties of certain clinically 

relevant drugs, such as mycophenolic acid,12) methotrexate,6) 

irinotecan,13) and pravastatin.14) The under-

lying mechanisms have yet to be elucidated; however, 

it is possible that this variant changes with protein ex-

pression and/or mRNA stability.

A number of sequence variations has been identified in 

ABCC2, but little is currently known about changes in the 

PK/PD profiles of substrate drugs in DJS patients. Based 
on its localization in human tissue, functionally sig-
nificant variation in the ABCC2 gene is expected to be 

associated with change in hepatic elimination and intestinal 

absorption of substrate drugs. Hulot et al.6) reported an 

unusual PK profile, mainly characterized by a three-fold 

reduction in the methotrexate elimination rate in a DJS 

patient receiving a high-dose methotrexate infusion for 
large B-cell lymphoma, resulting in severe methotrexate 

over-dosing and reversible nephrotoxicity. The present 

patient had a heterozygous mutation replacing a highly 

conserved arginine by glycine in the cytoplasmic part of 

the second membrane-spanning domain (position 412 of 

the ABCC2 gene).

In conclusion, we identified two SNPs and a large-
scale deletion, including exon 7, in a DJS patient. The 

frequencies of these two SNPs are relatively high in most 

ethnic populations; however, the deletion mutation is 

novel and may cause DJS.

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