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Genomic Structure of the Canalicular Multispecific Organic Anion–Transporter Gene (*MRP2/cMOAT*) and Mutations in the ATP-Binding–Cassette Region in Dubin-Johnson Syndrome

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Summary

Dubin-Johnson syndrome (DJS) is an autosomal recessive disease characterized by conjugated hyperbilirubinemia. Previous studies of the defects in the human canalicular multispecific organic anion transporter gene (*MRP2/cMOAT*) in patients with DJS have suggested that the gene defects are responsible for DJS. In this study, we determined the exon/intron structure of the human *MRP2/cMOAT* gene and further characterized mutations in patients with DJS. The human *MRP2/cMOAT* gene contains 32 exons, and it has a structure that is highly conserved with that of another ATP–binding–cassette gene, that for a multidrug resistance–associated protein. We then identified three mutations, including two novel ones. All mutations identified to date are in the cytoplasmic domain, which includes the two ATP-binding cassettes and the linker region, or adjacent putative transmembrane domain. Our results confirm that *MRP2/cMOAT* is the gene responsible for DJS. The finding that mutations are concentrated in the first ATP-binding–cassette domain strongly suggests that a disruption of this region is a critical route to loss of function.

Introduction

Dubin-Johnson syndrome (DJS) (MIM 237500), originally described in 1954 (Dubin and Johnson 1954), is an autosomal recessive disorder characterized by a defect in the transfer of endogenous and exogenous anionic conjugates from hepatocytes into the bile. This defect results in predominantly conjugated hyperbilirubinemia and a characteristic secondary rise of intravenously injected sulfobromophthalein in the blood plasma, after conjugation of sulfobromophthalein with glutathione in hepatocytes and transport of the conjugates from the hepatocytes to the blood (Dubin and Johnson 1954; Sprinz and Nelson 1954; Shani et al. 1970; Kondo et al. 1974). Although this syndrome is rare (Takino et al. 1977), cholestasis is frequently induced by many kinds of drugs (Farrell 1997; Sasabe et al. 1998) and is also observed in pregnant women and newborns. The putative transporter for conjugated bilirubin is also expected to export other conjugated substrates, including drugs, through the canalicular membrane. Identification and analysis of the transporters provide valuable information for understanding the pharmacokinetics of the drugs. Moreover, the analysis and understanding of DJS should lead to a better diagnosis and treatment of these clinical problems.

Hepatobiliary excretion of conjugated bilirubin is mediated by an ATP-dependent transport system, a canalicular multispecific organic anion transporter (cMOAT) (MIM 601107), in the apical (canalicular) membrane of hepatocytes (Kitamura et al. 1990; Oude Elferink et al. 1990, 1995). The multidrug resistance–associated protein (MRP), another member of the ATP-binding cassette (ABC)–transporter superfamily, can also transport glutathione conjugates (leukotriene C_4 and dinitrophenyl glutathione) (Cole et al. 1992; Loe et al. 1996; Keppler et al. 1997). These glutathione conjugates are also putative substrates for transport by the cMOAT protein (Cole et al. 1992; Loe et al. 1996), but the expression of the *MRP* gene in the liver is very low (Cole et al. 1992).

We previously had isolated the human *MRP2/cMOAT* gene as the candidate transporter for the glucuronide-

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Table 1

Mutations in *MRP2/cMOAT* and Serum Total- and Direct-Bilirubin and Urinary Coproporphyrine Isomer I Fractions, in Patients with DJS and in Their Families

Pedigree and Patient/ Family Member	Alteration in *cMAOT*	Exon	Putative Consequence	Concentration [Normal Range][a] T-bilirubin [.3–1.0] (mg/dl)	D-bilirubin [.1–.6] (mg/dl)	Urinary Coproporphyrin I Fraction [<27][b] (%)
1:						
DJ1	2302(C→T)/2302(C→T)	18	R768W/R768W	5.0	3.8	NT
2:						
DJ2	2302(C→T)/wild type	18	R768W/wild type	NT	NT	42.1
DJ3	2439+2(T→C)/wild type	18	Splice donor/wild type	NT	NT	43.5
DJ4	2302(C→T)/2439+2(T→C)	18	R768W/splice donor	1.3	.8	94.5
DJ5	2302(C→T)/2439+2(T→C)	18	R768W/splice donor	1.3	.8	93.6
DJ6	Wild type/wild type	…	Wild type/wild type	NT	NT	NT
3:						
DJ7	1815+2(T→A)/1815+2(T→A)	13	Splice donor/splice donor	5.2	3.8	NT
4:						
DJ8	2302(C→T)/2302(C→T)	18	R768W/R768W	4.8	3.2	NT
5:						
DJ9	2439+2(T→C)/4145(A→G)	18/29	Splice donor/Q1382R	2.5	1.6	80.0
6:						
DJ10	2439+2(T→C)/2439+2(T→C)	18	Splice donor/splice donor	2.1	1.6	85.7
DJ11	2439+2(T→C)/wild type	18	Splice donor/wild type	.9	.4	48.0
DJ12	2439+2(T→C)/wild type	18	Splice donor/wild type	.5	.2	36.9

[a] NT = not tested.
[b] Source: Frank et al. (1990).

and glutathione-conjugated antitumor agents (Taniguchi et al. 1996) and had found that it is highly homologous to the human *MRP* gene (Büchler et al. 1996; Taniguchi et al. 1996; Kool et al. 1997). These results, together with both the liver-specific expression of the gene and its mapping to chromosome 10q24 (Taniguchi et al. 1996), suggest that it may be causative in DJS. Animal models of DJS show a defect in the rat *MRP2/cmoat* gene (Büchler et al. 1996; Paulusma et al. 1996; Ito et al. 1997). A putative cMOAT protein cross-reacting with the antibody against human *MRP* at the canalicular membrane in the liver has not been observed in DJS (Kartenbeck et al. 1996). Patients with DJS show DNA sequence variations in the human *MRP2/cMOAT* gene (Paulusma et al. 1997; Wada et al. 1998).

In the present study, we searched for additional mutations in the *MRP2/cMOAT* gene in patients with DJS, to complete a genotype-phenotype correlation. We first determined the genomic structure of the human *MRP2/cMOAT* gene and then used this information to identify mutations in the genomic DNA of patients with DJS. Our combined studies indicate that, except for one, all the mutations identified are in the cytoplasmic domain containing the first ATP-binding domain and the adjacent transmembrane domain.

Subjects and Methods

Subjects

Three patients with DJS (DJ8–DJ10) were analyzed in this study. DJ8 is an 81-year-old Japanese man who has been jaundiced since childhood. DJ9 is a 24-year-old Japanese woman with a history of mild jaundice. DJ10 is a 26-year-old Japanese woman. DJ11 and DJ12 are the father and mother, respectively, of DJ10 and are not icteric and have had no abnormal laboratory findings, except in the case of the urinary coproporphyrin isomer I fraction (table 1). Findings from liver biopsy and/or urinalysis were used for diagnosis. Light-microscopy examination of the liver specimens of all patients showed that most of the hepatocytes contained pigment granules. DJ8 underwent laparoscopic examination, which showed that his liver was black. Table 1 shows the plasma bilirubin concentration and urinary coproporphyrin I fraction of these patients with DJS. Other laboratory findings for these three patients were within normal limits. The serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyltranspeptidase, lactate dehydrogenase, and α-fetoprotein were normal. Serum antibody titers showed no evidence of infection with rubella virus, cytomega-

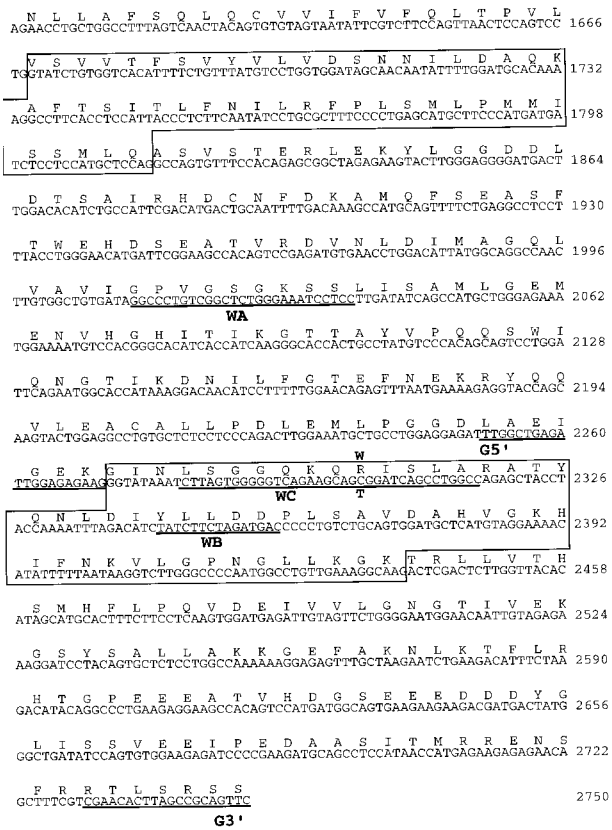

Figure 1 Nucleotide sequence of *cMOAT,* around the proximal ABC region. The nucleotide sequences of the primer pair G are indicated by thick underlines and boldface "G5′" and "G3′." Walker A, Walker B, and active transporter-family signature (Walker C) motifs are indicated by underlines and boldface "WA," "WB," and "WC," respectively. The 2302(C→T) transition detected in our previous and/or present study is indicated by a boldface "T" below the wild-type sequence Walker C. Amino acid change W is also indicated, as a boldface "W," above wild-type amino acid R. The deletion regions generated by splicing mutations 1669del147 (in DJ7), 2272del168 (in DJ3, DJ4, DJ5, DJ9, and DJ10) are circumscribed by boxes. Numbers on the far right indicate nucleotide positions, numbered from the translation start site.

lovirus, hepatitis B virus, or herpes simplex. All human samples were obtained under a protocol approved by the institutional review board, with all subjects providing informed consent.

Reverse Transcriptase-PCR (RT-PCR)

For RT-PCR, primer pairs G (5′-TTGGCTGAGATTGGAGAGAAG-3′ and 5′-GAACTGCGGCTAAGTGTTCG-3′, for G5′ and G3′, respectively, in fig. 1) and K (5′-GGCTGTTGAGCGAATAACTG-3′ and 5′-GCCTTCCAAATCTCCTCATC-3′) were used. Total RNA from peripheral blood leukocytes and the liver tissue of patients with DJS was isolated by use of ISOGEN (Nippongene). First-strand synthesis from total RNA was performed by use of random hexanucleotide primers and Moloney murine leukemia–virus reverse transcriptase (GIBCO BRL). The single-stranded cDNA was amplified by PCR with 2 pmol each of forward and reverse primer, as described above, by use of Ampli*Taq* Gold™ DNA polymerase (Perkin-Elmer). For PCR, 40 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 45 s) were performed.

Amplification of Genomic DNA

Samples of genomic DNA were prepared from peripheral blood leukocytes by standard methods, and table 2 shows the nucleotide sequences of the primers. The primer pairs used to amplify the genomic fragments containing each exon and splice junction were designed by comparison of the *cMOAT* cDNA sequence (Taniguchi et al. 1996; GenBank) with the partial genomic sequence determined in the present study (see Results). With these primers for PCR, 40 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 45 s) were performed.

Sequencing and Identification of Mutations

We sequenced PCR or RT-PCR products, either directly or after the subcloning, by using a Big-Dye Terminator Cycle Sequencing kit (Applied Biosystems) and a DNA sequencing system (model 377; Applied Biosystems). Both sense and antisense strands were sequenced, for confirmation.

Results

Genomic Organization of the Human MRP2/cMOAT *Gene*

We obtained nine clones by screening a human placental genomic library in λEMBL3, using, as probes, cDNA fragments covering the entire region of human *MRP2/cMOAT.* Partial sequencing of these clones by means of primers designed from and compared with the *MRP2/cMOAT* cDNA sequence (Taniguchi et al. 1996; GenBank) indicated that the human *MRP2/cMOAT* gene spans ⩾200 kb of genomic DNA containing 32 exons (fig. 2). All exons were flanked by the dinucleotides GT and AG, consistent with the consensus sequences for splice junctions in eukaryotic genes (Breathnach and Chambon 1981) (table 3). Exon 1 contained a *5′* UTR and 33 bp of coding sequence, whereas exon 32 consisted of a 130-bp coding sequence and a 3′ UTR. The exon-splitting pattern of the *MRP2/cMOAT* gene, especially for the ABC, the adjacent region, and the posterior half of the gene, was similar to that in the human *MRP* gene (Grant et al. 1997) (fig. 2), suggesting a close

Table 2

Sequences of PCR Primer Pairs Used to Amplify Genomic DNA

Primer Pair	Primer 1	Primer 2	Amplified Exon
M1G/1	5′-TTGTTGGCCAGCTCTGTTG-3′	5′-GTTCTTGTTGGTGACCACCC-3′	1
M1G/2	5′-AAAGCAGTGGGATGTGCTG-3′	5′-TGTCTCTACTGTGCACCAAGG-3′	2
M1G/3	5′-CACCGGAAACCATTCTGTTC-3′	5′-TTTGCCTCACTATGGATCCC-3′	3
M1G/4	5′-TCACGACAGTCTCCTCCCTC-3′	5′-CCAAAGGAAGTCTACATGGCC-3′	4
M1G/5	5′-ACATGAGGAGGTACCATGGG-3′	5′-GGATCCAGCCAATCCAATAC-3′	5, 6
M1G/6	5′-GGTGGAGATAGCCTCTGACC-3′	5′-TGCACTGAGAAGTATGAAGTGC-3′	7
M1G/7	5′-CCTGTACAGAGAAGGCCACG-3′	5′-CGGTCTTCATGACACAATGC-3′	8
M1G/8	5′-GGCTTTGGACAATTCTGGTC-3′	5′-TCCACCCATTGTCTGTGAAC-3′	9
M1G/9	5′-AGGCAAGAAGTCACAGTGCC-3′	5′-TTGCCCAAACTCCCATTAAG-3′	10
M1G/10	5′-ACAGTCAGGCAAGGGCTATG-3′	5′- TCCTTACCCACAGAGAGCC-3′	11
M1G/11	5′-ATCAGATACACCTGGTGCCC-3′	5′-TCTTGCGGTCAGGCCTANAG-3′	12
M1G/12	5′-GGAGGCTGGATGATCCTTAAG-3′	5′-TAGCAGTGAGAATGCCGACC-3′	13
M1G/13	5′-TGATCCTTAAGGCGCCTTC-3′	5′-CTGCAGGTCAACGATCTCTC-3′	14
M1G/14	5′- CTGATGGAGAAAGCGGAGAG-3′	5′-CATGGCAAATCCAGTATCAGG-3′	15
M1G/15	5′-CCCTGCTATCTCCTTCAAAGAC-3′	5′-GCTGAAATGGGAAGGAGAATC-3′	16
M1G/16	5′-GATCCTCAGTCATCCTGATGC-3′	5′-ACATGGAATATTGTGGCCAC-3′	17
M1G/17	5′-TCACAGGGTGACAAGCAAC-3′	5′-TTTACCATTCCACCCATGGC-3′	18, 19
M1G/18	5′-GTGTCTCCCTAGTCCATGATGG-3′	5′-TCACTCAGCTGGCATCAAAG-3′	20
M1G/19	5′-TGACTGTGACATCTGCTTGC-3′	5′-GGACAGAGGACATATTGCTCC-3′	21
M1G/20	5′-TCCACTTTCTCCTTGTGGTTG-3′	5′-CCTCCCTGTCAGAAAGATCC-3′	22, 23
M1G/21	5′- AAGGAGTTCTGGGAACACCAC -3′	5′- TTCTCAGGAAAGAGGCTGGG -3′	24
M1G/22	5′-GGAGCCTCTCATCATTCTGC-3′	5′-TTTCACACCACTAGCCATGC-3′	25
M1G/23	5′-GAGGCATTGCCTAAGAGTGC-3′	5′-AAAGATGGAGCCAGGGTTTG-3′	26
M1G/24	5′- TTGGTTTCTGTGCCTATGATG -3′	5′- AATGATGAAGGCTTAGGGCC -3′	27, 28
M1G/25	5′-AGAGATGGAGTAGCCAGTCAC-3′	5′-CAGCCACAAATGCATATTACC-3′	29
M1G/26	5′-AAGCTCAACCACAAACCAGC-3′	5′-ATTCTATGCCAGGCATCACC-3′	30
M1G/28	5′- CCTGCGTCTTTCCTTGGTC-3′	5′-AAACCTTCTGCCATCAGGTG –3′	31
M1G/27	5′-CCTTGTTTCAGGGTAATGGTC-3′	5′-CAGGACAGTGGTTGTCCC-3′	32

evolutionary relationship between these ABC-transporter genes.

Mutation Detection in the Human MRP2/cMOAT *Gene in Patients with DJS*

To identify mutations in the *MRP2/cMOAT* gene in patients with DJS, we designed primer pairs (see Subjects and Methods) (table 2) that cover the entire *MRP2/cMOAT* cDNA and exon-intron junction regions. By using PCR and DNA-sequence analysis, we analyzed the *MRP2/cMOAT* genes from three patients, DJ8–DJ10, and from the parents of DJ10 (DJ11 and DJ12). These patients are unrelated to each other. The first alteration was a homozygous missense mutation 2302(C→T) in DJ8 (fig. 1 and table 1), which has been identified in three other patients—DJ1, DJ4, and DJ5 (Wada et al. 1998)—who are unrelated to DJ8. Both children, DJ4 and DJ5, are heterozygous for the mutation. This alteration was accompanied by an amino acid substitution, R768W, in the active transporter-family signature (Higgins 1992) (fig. 1).

In DJ10 we identified the homozygous mutation 2439+2(T→C), a T→C transition of two bases after the 3′ boundary of the exon 18 (fig. 1 and table 1). This second mutation was also observed in patients DJ4 and DJ5 and in their mother, DJ3 (Wada et al. 1998) (table 1). The direct sequence of the genomic DNA in DJ10 and in her parents, DJ11 and DJ12, showed a heterozygous state for the mutation in DJ11 and DJ12, as well as perfect cosegregation of the mutation with the DJS trait (table 1). The mutation 2439+2(T→C) was found at the conserved splice-donor site, and we predicted that this mutation would cause a deletion of 168 nucleotides, at nucleotide positions 2272–2439, in cDNA (see below).

In DJ9 we identified two mutations, 2439+2(T→C), the same as the mutation identified in DJ10, and an A→G transition at nucleotide position 4145 (table 1). The 2439+2(T→C) mutation was identified in DJ9. These second and third mutations are heterozygous in DJ9. The third alteration resulted in amino acid substitution Q1382R in the position at the ABC region. The base substitutions 2302(C→T), 2439+2(T→C), and 4145(A→G) were not detected in any of 50 unrelated normal volunteers.

mRNA Analysis for Splice-Site Mutations

We confirmed the mutations 2302(C→T) and 4145(A→G) in the *MRP2/cMOAT* cDNA in DJ8 and DJ9, respectively, by RT-PCR and DNA sequencing us-

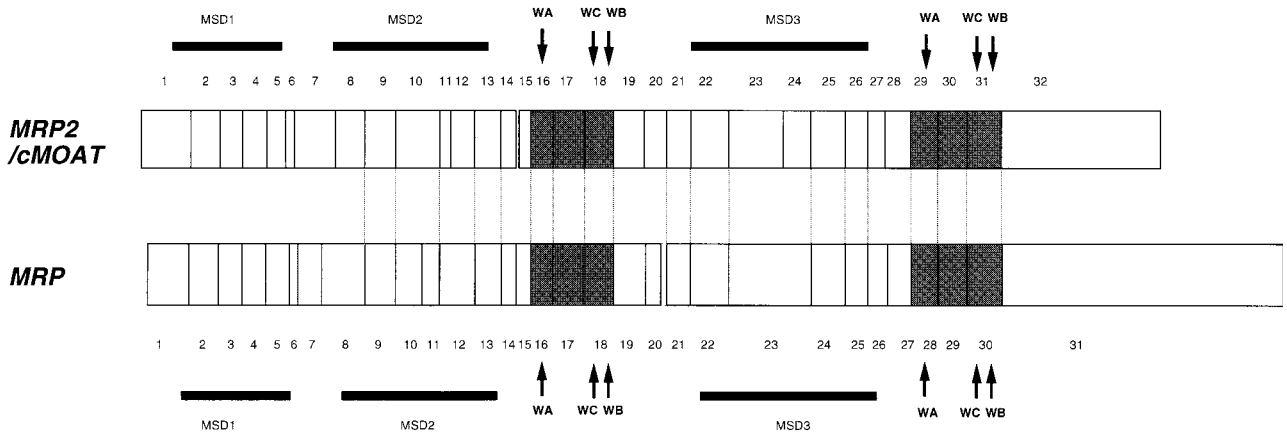

Figure 2 Comparison of exon-splitting pattern, between *MRP2/cMOAT* and its related gene, *MRP* (Grant et al. 1997). Exons are represented by unshaded boxes, and splice sites are indicated by vertical lines. The exon number for each gene is shown both above and below. The highly conserved motifs—Walker A, Walker B, and Walker C—in an ABC are denoted as "WA," "WB," and "WC," respectively. Shaded boxes indicate the exons coding the ABC. Predicted membrane-spanning domains are indicated by horizontal bars (MSD1, MSD2, and MSD3) (Deeley and Cole 1997; Hipfner et al. 1997; Tusnady et al. 1997). Regions in which the two genes show exactly identical splitting are indicated by vertical dashed lines.

ing primer pairs G and K (see Subjects and Methods) and mRNA extracted from peripheral blood lymphocytes (data not shown). To determine whether the 2439+2(T→C) mutation might cause splicing alteration and deletion of the *MRP2/cMOAT* gene, lymphocyte RNA from DJ10 was isolated and analyzed by RT-PCR. A 500-bp product was detected in control individuals, whereas a 332-bp product was detected in DJ10 by RT-PCR analysis using primer pair G (see Subjects and Methods and fig. 1). Sequence analysis of the 332-bp product showed the expected deletion of 168 nucleotides (fig. 1).

Discussion

We previously had reported mutations of the *MRP2/cMOAT* gene in patients with DJS and had suggested that the *MRP2/cMOAT* gene is responsible for the defects in patients with DJS (Wada et al. 1998). In the present study, we have identified other mutations, including two new ones. The first mutation, identified in DJ8 in the present study, is 2302(C→T) and is associated with the amino acid change Arg^{768}→Trp (R768W) in the highly conserved domain, the Walker C motif. This mutation is the same as that which we previously had identified in patients DJ1, DJ4, and DJ5. Patients DJ4 and DJ5 are brothers, but the other patients are not related to each other. Among the seven patients analyzed, this mutation was identified in four individuals, suggesting the presence of a founder effect. The second mutation is a homozygous T→C transition in patient DJ10, found two bases after the 3′ boundary of exon 18 (the splice-donor site). The third mutation is 4145(A→G), which predicts amino acid change Gln^{1382}→Arg (Q1382R) within the ABC at the carboxyl-terminal end. This same amino acid substitution in the ABC is in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) in patients with cystic fibrosis (Dörk et al. 1994), suggesting that this mutation could affect the function of *MRP2/cMOAT*. These second and third mutations were in a compound-heterozygous state in DJ9.

Interestingly, all the mutations observed in the patients with DJS were localized within the ABC or its adjacent regions. This tendency might be due to the absence of any functional effect in any mutation occurring outside the ABC and its adjacent domain for cMOAT, leading to no alteration of phenotype. The existence of several polymorphisms in other domains (i.e., besides the ABC region) of other ABC transporters, such as CFTR (Cystic Fibrosis Mutation Data Base) or ABCR (Allikmets et al. 1997), is consistent with this possibility. With the *CFTR* gene, ~80% of mutations in patients are identified within the ABC region, and almost all of them cause a severe form of the disease (Welsh and Smith 1993), suggesting that alteration in the ABC could impair the transporter activity. Site-directed mutagenetic analysis of P-glycoprotein, which is another member of the ABC-transporter superfamily, showed that the transmembrane domain is important for substrate specificity but not for the transporter activity itself (Gottesman et al. 1995; Taguchi et al. 1997). Mutations localized around the ABC thus might disrupt the function of the transporter, not only in CFTR and P-glycoprotein but also in MRP2/cMOAT. By contrast, mutations in the transmembrane domain could change the specificities for their substrates but might not completely disrupt their trans-

Table 3

Exon-Intron Organization of the Human *cMOAT* Gene

Exon	3′ Acceptor	Exon 5′ End	Exon Size (bp)	Exon 3′ End	5′ Donor
1		Not determined	5′ UTR+33	TTTTGG	gtgaga
2	ctccag	AATTCC	174	AAGCAG	gtaaag
3	tctcag	GTATTC	126	ACATGG	gtaaga
4	ctccag	CTCCTG	135	TTACAG	gtaagg
5	tcatag	GGTGAC	108	TCAAAT	gtgaga
6	tttcag	AATCCA	56	TGACAG	gtagga
7	ttccag	CATCAT	235	GTCCTG	gtaact
8	acctag	GAAGAT	164	GCTGAA	gtgagt
9	tggcag	ATTGCT	178	AAGAAG	gtaagc
10	ctttag	GCATTG	255	ATTCAG	gtaaag
11	tggcag	GTCAAA	66	ATCAAG	gtgaga
12	tattag	ATCCTG	138	GTCCTG	gtgagt
13	ttccag	GTATCT	147	CTCCAG	gtaggt
14	ctctag	GCCAGT	85	ATTTTG	gtaaat
15	caacag	ACAAAG	67	CCGAGA	gtgagt
16	tttcag	TGTGAA	127	ATCAAG	gtgaga
17	atctag	GGCACC	177	GAGAAG	gtactt
18	cttcag	GGTATA	168	GGCAAG	gtgaga
19	ttatag	ACTCGA	181	CCACAG	gtatgt
20	ccgcag	TCCATG	127	CCGCAG	gttggc
21	tctcag	TTCTAG	136	GGAAAG	gtgaac
22	ttgcag	GTGAAG	220	CCCAAG	gtatgt
23	ctctag	GTATAT	155	GCCGGC	gtaagt
24	tcctag	GATATT	156	GTTCAG	gtaggt
25	gtccag	ATGTTT	200	CAACAG	gtgagg
26	ccacag	GTGGCT	127	CTCAAT	gtgagc
27	ctgtag	ATCACA	102	AATGAG	gtaagg
28	tgccag	GCACCC	144	GAGAAG	gtaggt
29	tcgcag	ATTGGT	159	CCCCAG	gtgagc
30	ttgcag	GACCCC	167	CCTGAG	gtaatg
31	ctgcag	CATAGG	195	TGACAA	gtgagt
32	tttcag	GGTAAT	130+3′ UTR	Not determined	

NOTE.—Exon sequences and intron sequences are shown as uppercase letters and lowercase letters, respectively.

porter activities. However, further identification of mutations in MRP2/cMOAT is necessary to confirm this possibility.

Table 1 summarizes the mutations that we identified in the patients with DJS. The serum bilirubin concentration and the proportion of urinary coproporphyrin isomer I in these patients have been determined. It will be important to learn whether the level of bilirubin concentration is dependent primarily on each specific mutation. According to the report by Kondo et al. (1974), the serum bilirubin concentration in 40 patients with DJS was 1.3–6.9 mg/dl. We identified homozygous mutation 2302(C→T) in DJ1 and DJ8, whose bilirubin concentration was 5.0 mg/dl and 4.8 mg/dl, respectively, concentrations near the higher end of the range described by Kondo et al. (1974). This mutation is accompanied by an amino acid substitution, R768W, in the Walker C motif, which is a highly conserved domain among the ABC-transporter family. This mutation might cause severe disruption of the transporter activity and, consequently, shows a relatively high serum bilirubin concentration in DJS. A high bilirubin level similar to that observed in DJ1 and DJ8 was observed in DJ7, whose mutation in *MRP2/cMOAT* was 1815+2(T→A). This mutation causes abnormal splicing and, consequently, a 147-bp deletion in *MRP2/cMOAT* cDNA, without an immature stop codon. This deletion of the ABC region likely reduces the level of normal cMOAT protein and its activity. The absence of cMOAT in the liver of DJ7 was confirmed by immunohistochemical analysis (data not shown).

The urinary coproporphyrin isomer I fraction was examined in several patients and their family members. All of the patients with DJS showed >80% of urinary coproporphyrin isomer I fraction, whereas normal controls showed <27% (Frank et al. 1990). Interestingly, all examined family members who carry a heterozygous mutation in the *MRP2/cMOAT* gene (i.e., individuals DJ2, DJ3, DJ11, and DJ12) showed the normal range of T- and D-bilirubin levels, whereas they showed levels of urinary coproporphyrin isomer I fraction that were slightly higher than the normal range of <27% (table

1). The mechanisms for abnormal coproporphyrin isomer I fraction in the urine are unknown, but a correlation may exist between the urinary coproporphyrin isomer I level and the homozygous/heterozygous status of mutation in the *MRP2/cMOAT* gene. In Japan, the expected number of people carrying a heterozygous mutation in *MRP2/cMOAT* is ⩾200,000, which is calculated on the basis of the frequency of patients with DJS—that is, 121/100,000,000 people in a nationwide survey in Japan (Takino et al. 1977; Vogel and Motulsky 1997, pp. 129–162). The transport and/or pharmacokinetics of some substrates might be affected by a heterozygous mutation in the *MRP2/cMOAT* gene, and a putative differential responsiveness to some drugs and their side effects in these carriers might be also an important clinical factor. Further study is needed for an understanding of how *MRP2/cMOAT* maintains a steady state of the levels of bilirubin and coproporphyrin isomer I in patients with DJS.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cystic Fibrosis Mutation Data Base, http://www.genet.sickkids.on.ca/cftr/

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for the human *MRP2/cMOAT* gene [U63970])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for DJS [MIM 237500] and cMOAT [MIM 601107])

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