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REVIEW

Coen C. Paulusma · Ronald P.J. Oude Elferink

The canalicular multispecific organic anion transporter and conjugated hyperbilirubinemia in rat and man

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Abstract The human Dubin-Johnson syndrome is an autosomal recessive liver disease characterized by a chronic conjugated hyperbilirubinemia. Patients have impaired hepatobiliary transport of many endogenous and xenobiotic compounds. A similar disease phenotype has been described for a naturally occurring mutant Wistar rat strain, the TR⁻ rat, which is defective in the, functionally defined, canalicular multispecific organic anion transporter (cMOAT). The complementary DNA encoding this protein has been cloned from rat and recently from human liver. cMOAT is a new member of the ATP-

binding cassette transporter family, and homologous to the multidrug resistance-associated protein 1. A mutation in the *cMOAT* gene is responsible for the phenotype observed in TR⁻ rats. This information should soon lead to a complete genetic characterization of the human Dubin-Johnson syndrome.

Key words Hyperbilirubinemia · Multidrug resistance · Organic anions · Bilirubin · Liver · Bile · ATP-binding cassette transporter

Abbreviations *B-UGT* Bilirubin UDP-glucuronosyltransferase · *BSP* Bromosulphophthalein · *cBAT* Canalicular bile acid transporter · *cMOAT* Canalicular multispecific organic anion transporter · *EHBR* Eisai hyperbilirubinemic rat · *GS-BSP* Bromosulphophthalein-S-glutathione · *GS-DNP* Dinitrophenyl glutathione · *GY* Groningen yellow rat · *MRP* Multidrug resistance-associated protein · *NTCP* Na⁺-dependent taurocholate protein · *OATP* Organic anion transporting polypeptide · *PCR* Polymerase chain reaction · *PKC* Protein kinase C

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Introduction

The liver plays a major role in the detoxification of many endogenous and xenobiotic compounds, a process in which bile formation is essential. The major constituents of bile are bile acids; also present are phospholipids, cholesterol, and endogenous and xenobiotic compounds. Detoxification is a multifactorial process which encompasses uptake, intracellular metabolism, and secretion of toxic compounds. Detoxification takes place in the hepatocyte and is accomplished by a diversity of transferase-mediated conjugation reactions with charged moieties such as glutathione, glucuronide, and sulfate, resulting in negatively charged, amphiphilic compounds that are efficiently secreted into bile or urine. The hepatocyte is an epithelial cell which comprises three membrane domains, the basal, lateral, and apical (or canalicu-

lar) membrane. Formation of primary bile takes place in the bile canaliculi, the space formed by two adjacent hepatocytes, which represent the most proximal part of the biliary tree; water flow into the canaliculus is driven primarily by bile salt secretion, which creates an osmotic pressure in the canalicular lumen, resulting in water flow through the tight junctions.

The lateral domain of the basolateral membrane contains a wide variety of transport proteins which are involved in the hepatic uptake of potential toxic compounds. Two transporters involved in the uptake of bile salts and other organic anions have been cloned from rat and human liver, the Na^+ -dependent taurocholate protein (NTCP) [1, 2], and the Na^+ -independent organic anion transporting polypeptide (OATP) [3, 4] (Fig. 1). Expression studies with the cDNAs encoding these proteins have indicated that NTCP is involved primarily in the sodium-dependent uptake of conjugated bile salts such as taurocholate and also in that of unconjugated bile salts such as cholate [1, 2, 5, 6], while the OATP transports a wide variety of structurally unrelated amphiphilic organic anions including bromosulphophthalein (BSP), taurocholate and cholate [3, 4, 7], anionic and neutral steroid conjugates, and some amphiphilic organic cations [8]. After intracellular modification these compounds are efficiently secreted into bile by canalicular transport proteins. During the past few years substantial progress has been made toward identifying proteins involved in the formation of primary bile.

Several ATP-dependent transport systems have been identified in this membrane domain, including the canalicular bile acid transporter (cBAT), and the canalicular multispecific organic anion transporter (cMOAT), which mediate the active transport of bile salts and nonbile salt organic anions, respectively (reviewed in [9, 10]). In addition, ATP-dependent transporters are present for the extrusion of cationic and neutral amphipathic compounds (MDR1 P-glycoprotein) and for phospholipids (MDR2/3 P-glycoprotein; reviewed in [10]; Fig. 1). The identification of a transport-deficient mutant Wistar rat strain, the TR^- rat [5] has contributed to the functional characterization of the cMOAT protein [11]. These rats have an autosomal recessive defect in the hepatobiliary excretion of a wide variety of negatively charged, amphiphilic organic anions. TR^- rats have a similar phenotype as patients with Dubin-Johnson syndrome; this syndrome is characterized by a chronic conjugated hyperbilirubinemia which results in jaundice and is caused by impaired hepatobiliary excretion of many organic compounds [12, 13].

We have recently isolated a cDNA from rat liver [14] which encodes a new member of the ATP-binding cassette (ABC) transporter family [15]. Based on the absence of the gene product, and a 1-nucleotide deletion in the transcript of this gene in the TR^- rat we have concluded that we isolated the cDNA which encodes the cMOAT protein. This review describes the current knowledge on two major transporters involved in bile formation, cBAT and cMOAT, the isolation of the cMOAT cDNA, and the implications of this finding for the human Dubin-Johnson syndrome.

Fig. 1 Schematic representation of a hepatocyte including a bile canaliculus emphasizing several important transport proteins involved in bile formation. OATP, organic anion transporting polypeptide; OA, organic anion; NTCP, Na^+ -dependent taurocholate protein; BS, bile salt; cBAT, canalicular bile salt transporter; cMOAT, canalicular multispecific organic anion transporter; MDR1, multidrug resistance protein (MDR) P-glycoprotein 1; MDR2/3, MDR2/3 P-glycoprotein

Hepatobiliary transport of bile salt and nonbile salt organic anions

The two major transport proteins which are involved in canalicular organic anion secretion are cBAT and cMOAT. The phenotype of the human Dubin-Johnson syndrome strongly suggests that cBAT and cMOAT are separate transport systems which mediate the hepatobiliary transport of bile salt and nonbile salt organic anions, respectively. Dubin-Johnson syndrome is a rare congenital autosomal recessive defect characterized by a chronic conjugated hyperbilirubinemia [12, 13]. In these patients it has been demonstrated that the hepatobiliary transport of bilirubin and other nonbile salt organic anions is severely impaired [16, 17]. Bile salt transport, on the other hand, is unimpaired in these patients [18], indicating that the hepatobiliary transport of bile salt and nonbile salt organic anions is mediated by two separate transport systems.

Canalicular transport of bile salt organic anions in rat

Canalicular bile salt transport was initially characterized as a membrane potential driven process [19–21]. The high bile salt gradient, however, could not be achieved solely by a single membrane potential driven transporter. Transport studies in canalicular, inside-out membrane vesicles from rat liver demonstrated strongly increased uptake of taurocholate in the presence of ATP [22–24]. In addition, ATP-dependent taurocholate uptake by these canalicular membrane vesicles was competitively inhibited by other bile salts including glycocholate [24, 25]; it has since been demonstrated that the electrogenic taurocholate transport in canalicular membrane vesicles was probably caused by a contamination of the canalicular membrane fraction with endoplasmic reticulum mem-

branes [26]. Further purification of the canalicular membrane fraction revealed a complete loss of electrogenic taurocholate transport in the enriched canalicular fraction, while the ATP-dependent taurocholate transport remained. The electrogenic transport activity resided in the endoplasmic reticulum enriched fraction. These data suggest that the hepatobiliary excretion of taurocholate and other bile salt organic anions is solely accomplished by an ATP-dependent transport system, while electrogenic bile salt transport is restricted to the endoplasmic reticulum. The nature and function of the latter transport system remains to be established.

Several attempts have been made to isolate the protein which is responsible for the ATP-dependent transport of taurocholate. Müller et al. [23] identified a 110-kDa protein in the canalicular membrane fraction which after reconstitution in liposomes facilitated ATP-dependent taurocholate transport. Partial sequencing of this protein [27] revealed amino acid identity with the already cloned rat liver ecto-ATPase [28]. To determine whether both the ATPase and bile salt transport activities are regulated by a single 110-kDa protein the *ecto-ATPase* cDNA was transfected into COS cells [29]. After transfection these cells expressed a 110-kDa protein and revealed greater taurocholate transport activity than mock-transfected COS cells. Truncated *ecto-ATPase* cDNA did not confer taurocholate transport activity. Moreover, staurosporine-induced protein kinase C (PKC) activity inhibition abrogated the putative taurocholate transport completely but did not affect ATPase activity, suggesting that bile salt transport and ecto-ATPase activity are not coupled. Subfractionation experiments with canalicular membranes, however, demonstrated that the ecto-ATPase and taurocholate transport activities are two different entities [26]. In addition, sequence analysis of the ecto-ATPase predicts a single transmembrane domain [28], a configuration which is very uncommon for a transport protein. Taken together these data suggest that the major taurocholate transporting protein in canalicular membranes has not been identified yet. On basis of its functional characterization it may be assumed that this protein also is a member of the ABC transporter family.

Canalicular transport of nonbile salt organic anions in rat

cMOAT has been extensively characterized at the functional level, using mainly the TR⁻, a Wistar rat strain which lacks a functional, ATP-dependent transport system for nonbile salt organic anions (reviewed in [10]). TR⁻ rats have an autosomal recessive transport defect and are characterized by mild chronic conjugated hyperbilirubinemia, which results in jaundice [11]. Apart from the TR⁻ rat, two other transport-deficient rats were identified, the Groningen yellow rat (GY), a Wistar rat derived from the same colony as TR⁻ rats [30], and the Eisai hyperbilirubinemic rat (EHBR), a Sprague-Dawley strain [31, 32]. These animals have a defect in the hepatobiliary excretion of a broad range of organic anions, in-

Table 1 List of compounds [10] of which the hepatobiliary excretion is impaired in the transport mutant TR⁻/GY or EHBR rats.

Compound	Number of negative charges	Reference
Endogenous compounds		
Conjugated bilirubin	2	[39]
Glutathione, GSH	2	[37]
Glutathione, GSSG	4	[40]
Cysteinyl leukotrienes	2/3	[35]
Triiodothyronine glucuronide	2	[41]
Coproporphyrin I	2	[11]
Bile salt conjugates		
Cholate 3- <i>O</i> -glucuronide	2	[38]
Lithocholate 3- <i>O</i> -glucuronide	2	[38]
Nordeoxycholate 3- <i>O</i> -glucuronide	2	[42]
Tauro/glycolithocholate 3-sulfate	2	[30]
Taurochenodeoxycholate 3-sulfate	2	[30]
Nordeoxycholate 3-sulfate	2	[42]
Exogenous compounds		
Ceftriaxone	2	[43]
Ampicillin	2	[44]
Carboxydichlorofluorescein	2	[45]
Dibromosulphophthalein	2	[39]
Bromosulphophthalein glutathione	4	[39]
Dinitrophenyl glutathione, GS-DNP	2	[37]
Glutathionyl bromoisovalerylurea	2	[46]
Naphtol-1-glucuronide	1	[47]
Indocyanine green	2	[48]
Gadolinium ethoxybenzyl DTPA	2	[49]
Metals		
Zinc		[50]
Copper		[50]
Manganese		[51]

cluding bilirubin glucuronides [33, 34] and other multivalent organic anions such as glutathione-S-conjugates (e.g., leukotriene C₄ [35, 36]), the model organic anion dinitrophenyl glutathione (GS-DNP) [11, 37], and 3-*OH*-glucuronidated [38] and 3-*OH*-sulfated bile salts [30]. Apart from these substrates, a broad range of mainly bivalent endogenous and xenobiotic compounds have been identified by studying different experimental systems which include intact rats, perfused liver, isolated hepatocytes, hepatocyte couplets, and canalicular membrane vesicles (see Table 1, including references).

Furthermore, it has been demonstrated that the organic anion transport activity is stimulated either directly or indirectly by PKC. Activation of the PKC-pathway results in stimulated organic anion efflux from isolated hepatocytes while activation of the cyclic AMP pathway has no stimulatory effect. The PKC-mediated organic anion efflux is absent from TR⁻/GY rat hepatocytes, indicating that the PKC-mediated stimulatory effect is accomplished via the cMOAT protein [52].

Cross-breeding studies in which TR⁻ rats were crossed with GY [38] and EHBR rats [53] resulted in jaundiced offspring in both cases, which indicates that the same gene is affected in all three mutant strains. Furthermore, the genetic defect in TR⁻/GY rats is specific for liver since no impaired organic anion transport activi-

ty is observed in the intestine or kidney of these rats [47]. Other, organ-specific, transport systems are apparently involved in the intestinal and renal clearance of organic anions. Despite the genetic defect some residual organic anion transport activity has been observed in the transport mutant rats; where canalicular membrane vesicles from mutant rats lack ATP-dependent organic anion transport, they retained some membrane potential dependent transport of organic anions [54]. Jansen et al. [48] reported a preserved low-affinity transport pathway for some cMOAT substrates in TR-/GY rats. The exact nature of these additional organic anion transporting systems and their contribution to the bile salt independent bile flow remains to be determined.

Isolation of the rat cMOAT cDNA

Several attempts have been made to isolate the cMOAT protein. Pikula et al. [55, 56] have isolated a 90-kDa glycoprotein from rat liver plasma membranes which, upon reconstitution into liposomes, displays ATP-dependent organic anion transport activity. In addition, they demonstrated that this transport is stimulated by PKC-mediated phosphorylation of the reconstituted protein. This observation is in agreement with the data described by Roelofsen et al. [52], who demonstrated a PKC-mediated stimulation of organic anion transport in isolated hepatocytes. Furthermore, Pikula et al. [56] have shown that the ATP-ase activity of the 90-kDa protein is inhibited by vanadate, a compound which is known to inhibit ATP-dependent organic anion transport. The authors suggest that the 90-kDa protein is responsible for the multi-specific organic anion transport activity localized in the canalicular membrane of the hepatocyte. Their data, however, do not include evidence for a relation between the isolated 90-kDa protein and the transport defect observed in the TR-/GY rat.

Analogous to the identification of the P-glycoproteins, the clues about the identity of the cMOAT protein came from the cancer research field. Transport studies in plasma membrane vesicles isolated from cells overexpressing the human multidrug resistance-associated protein (hMRP) 1 [57], have demonstrated a role for hMRP1 in the ATP-dependent transport of the glutathione conjugates leukotriene C₄ and GS-DNP [58, 59]. The hMRP1 cDNA encodes a 1531 amino acid [57] member of the ABC transporter family which confers resistance to many structurally and functionally unrelated substrates (reviewed in [60]). Furthermore, it has been demonstrated that this MRP-mediated resistance is dependent on intracellular glutathione levels [61–64]. hMRP1 is expressed in all human tissues [65], with high expression in lung, spleen, thyroid, testis, adrenal gland, and gall bladder, and extremely low expression in liver [57, 65]. The physiological function of hMRP1 is at this moment still unknown.

Because of the striking resemblance in substrate specificity between hMRP1 and cMOAT *hMRP1* was consid-

ered a possible candidate gene for cMOAT [58, 59]. Several points argued against the idea of hMRP1 being identical to cMOAT. First, the hMRP1 expression in liver is very low, which is in contrast with the very high organic anion transport activity in this organ. Secondly, Evers et al. [66] demonstrated that the hMRP1 protein is routed predominantly to the lateral domain of the basolateral membrane when transfected into the (polarized) epithelial pig kidney cell line LLC-PK1, which renders it unlikely that hMRP1 is responsible for the hepatobiliary (apical) excretion of organic anions. Thirdly, and most importantly, the transport defect in the TR-/GY rat appears to be specific for the liver [47], whereas hMRP1 is expressed in all human tissues [65].

The cloning strategy for the rat *cMOAT* cDNA was based on the hypothesis that cMOAT is a liver-specific homologue of hMRP1 [14]. Indeed, the cDNA obtained encoding the cMOAT protein turned out to be homologous to the MRP1 protein; it is a 1541 amino acid transmembrane glycoprotein with a molecular weight of 200 kDa and an exclusive canalicular/apical localization [14], while the hMRP1 protein is made up of 1531 amino acids, has an apparent molecular weight of 190 kD and is localized in the lateral domain of the basolateral membrane [57, 66]. The cMOAT protein is highly expressed in liver and to a much lesser extent in kidney, ileum, and duodenum [14], while the MRP1 protein is expressed in all tissues [65]. As with hMRP1, cMOAT belongs to the ABC transporter family and shares highest sequence identity with the hMRP1 [57], the yeast cadmium factor 1 [67], a protein which confers cadmium resistance in yeast and is capable of transporting organic anions [68, 69], the cystic fibrosis transmembrane conductance regulator [70], a protein involved in chloride transport, and the rat sulfonylurea receptor [71], a protein which is postulated to regulate insulin secretion.

A report by Mayer et al. [72] related the absence of MRP1, or an isoform of MRP1 to the congenital transport defect in mutant TR-/GY rats based on two observations. By using an antibody raised against a synthetic peptide corresponding to amino acid residues 1517–1531 of the hMRP1 sequence they detected both a lateral and a canalicular localization of the rMRP1 gene product in Wistar liver, but only a lateral localization in TR-/GY rat liver. Apparently this antibody recognizes both rMRP1 and rat cMOAT. Furthermore, they could not amplify a polymerase chain reaction (PCR) fragment from TR-/GY rat liver using primers directed to the hMRP1 sequence, a fragment which could be amplified from Wistar rat liver. The sequence of this PCR fragment is identical to the rat *cMOAT* sequence [14]. Ito et al. [73] describe the absence of RNA transcripts in liver from mutant EHBR rats after hybridization with a PCR fragment amplified from Sprague-Dawley rat liver using degenerate primers directed to the hMRP1 sequence. They relate the lack of expression to the transport defect in EHBR rats and suggest that this PCR fragment encodes a part of the transport protein which is defective in these rats. This indeed

is the case since the PCR fragment is identical to the *cMOAT* sequence [14].

We have sequenced the complete *cMOAT* cDNA from TR-/GY rat liver and demonstrated a one-nucleotide deletion. This deletion results in a frameshift and subsequent introduction of a stop codon which is responsible for the absence of the gene product from the canalicular membrane. This mutation explains the congenital transport defect in these rats [14]. A recent study by Büchler et al. [74] reports the cloning of the canalicular isoform of the multidrug resistance protein (*cMRP*). *cMRP* was not expressed in liver of either TR-/GY or EHBR rats. Using a *cMRP*-specific polyclonal antibody they demonstrate the absence of the *cMRP* protein from the canalicular membrane of both mutant rats. Indeed, *cMRP* is identical to the *cMOAT* sequence. Very recently the complete *cMOAT* cDNA from Sprague-Dawley rats has been isolated, and sequence analysis of EHBR *cMOAT* cDNA demonstrates a one-nucleotide substitution resulting in the introduction of a stop codon [75].

Hereditary chronic hyperbilirubinemias in man

As mentioned above, the phenotype of TR-/GY rats closely resembles that of patients with the Dubin-Johnson syndrome. Other chronic inherited hyperbilirubinemias have been described in addition to Dubin-Johnson syndrome [76]. The most common are the unconjugated hyperbilirubinemias, in which unconjugated bilirubin accumulates in the circulation. Two forms have been described varying from mildly elevated serum bilirubin in Gilbert syndrome [77], to severely elevated serum bilirubin in Crigler-Najjar syndrome (type I) [78]. Both syndromes are caused primarily by a defect in the activity of bilirubin UDP-glucuronosyltransferase (B-UGT), the enzyme involved in the glucuronidation (detoxification) of bilirubin. For Gilbert syndrome a promoter abnormality has been identified which results in lowered expression of B-UGT [79]. In Crigler-Najjar type I patients no B-UGT activity was found, while Crigler-Najjar type II patients had low residual B-UGT-activity; several different mutations in the B-UGT gene have been described which are responsible for the decreased, or the lack of, B-UGT activity [80].

The other inherited hyperbilirubinemias are the conjugated hyperbilirubinemias, which are quite rare and clinically much less severe as the Crigler-Najjar syndrome. Two syndromes have been described which are characterized by high serum levels of, predominantly conjugated bilirubin: Dubin-Johnson's syndrome [12, 13] and Rotor's syndrome [81]. The genetic defect underlying the impaired hepatobiliary transport in Dubin-Johnson patients has not been identified yet, but the cloning of the human *cMOAT* cDNA will elucidate whether this gene is involved in Dubin-Johnson syndrome. Despite the fact that Rotor's syndrome phenotypically resembles Dubin-Johnson's syndrome they have shown to be distinct disorders (reviewed in [82]). The final part of this

review will focus on the biochemical features which underly the Dubin-Johnson syndrome and the relation with the *cMOAT* protein.

Dubin-Johnson syndrome

Dubin-Johnson syndrome is a rare and benign disorder first described in 1954 [12, 13]. The syndrome is frequently observed among Persian Jews (1:1300) [83] in whom it is often linked to coagulation factor VII deficiency [84, 85]. Another report describes the association of Dubin-Johnson syndrome with hemoglobin-O disease (sickle-cell anemia) in a Sudanese family [86]. The syndrome is characterized by a chronic, predominantly conjugated, nonhemolytic hyperbilirubinemia, caused by an impaired hepatobiliary transport system for nonbile salt organic anions in the canalicular membrane of the hepatocyte. Furthermore, liver histology is normal except for the syndrome-characteristic lysosomal accumulation of a black pigment. In addition, the urinary coproporphyrin excretion in these patients is abnormal.

The discovery of experimental animal models for Dubin-Johnson syndrome, including Corriedale sheep [87] and three more suitable transport-mutant rat strains, has contributed to the biochemical characterization of the transport system involved in this syndrome. Both Corriedale sheep [87, 88] and TR-/GY rats [11, 39, 48] show impaired secretion of bilirubin-glucuronide, bromosulphophthalein-*S*-glutathione (GS-BSP), and indocyanine green (among various other organic anions; Table 1), substrates which have shown to be transported by the *cMOAT* protein; bile salt transport is unaffected. In Dubin-Johnson patients the hepatobiliary transport of these nonbile salt organic anions is impaired as well; hepatic uptake and storage of these compounds is not affected, indicating that the defect in Dubin-Johnson patients is caused primarily by a defect in a canalicular transport system [16, 17, 89]. Recent studies on the transport of leukotriene C₄ (an endogenous *cMOAT* substrate in rats) in Dubin-Johnson patients demonstrate a defect in the hepatobiliary elimination of this substrate, leading to a compensatory urinary excretion of leukotrienes [90]. As in the mutant animals, serum levels of endogenous bile salts are normal, and the hepatobiliary transport of bile salts in these patients is unaffected [18, 91].

A phenomenon which is indicative for the Dubin-Johnson syndrome is the clearance spectrum of BSP from the serum. When BSP is administered to a patient, initial BSP levels in the serum are high. BSP is rapidly taken up by the liver, and this results, as in healthy persons, in a decrease in BSP serum levels. Detoxification of BSP is accomplished in the hepatocyte by a transferase-mediated conjugation with glutathione, which results in the formation of GS-BSP. The hepatobiliary transport of this substrate, however, is impaired in these patients, and it therefore leaks back into the circulation, while in controls it is quantitatively excreted into bile. This results in a typical secondary rise of serum BSP levels in

Dubin-Johnson patients [16, 92]. A similar secondary rise has been observed for bilirubin but not for organic anions, which are not conjugated in the hepatocyte [93].

An important phenotypic feature of the Dubin-Johnson syndrome is the lysosomal pigment accumulation in the hepatocyte [12, 13]. Subcellular fractionation experiments on liver biopsies demonstrate that lysosomes accumulate melanin [94]. A similar black hepatic pigmentation has been observed in Corriedale sheep [88, 95]. Injection of [^3H]epinephrine or its metabolite [^3H]metanephrine glucuronide, compounds which are normally excreted into bile, results in lysosomal accumulation of radioactivity [87]. The liver of TR-/GY rats is not black although sparse pigment accumulation is observed. Still, when TR-/GY rats receive a tryptophane-, tyrosine-, and phenylalanine-supplemented diet (precursors in the metanephrine synthesis), some lysosomal pigment accumulation is observed, as opposed to the case in control rats. In addition, injection of metanephrine results in a black liver which, however, is not associated with pigment accumulation [53].

The most important diagnostic tool for Dubin-Johnson syndrome is the abnormal urinary excretion of coproporphyrins, metabolic byproducts of the heme synthesis. Two coproporphyrin isomers exist in the circulation, coproporphyrin isomers I and III. Isomer III is a precursor in the synthesis of heme, while isomer I is a true byproduct in this pathway. The coproporphyrin isomers I and III leave the body by bile and urine, while the total coproporphyrin excretion into bile is three times the urinary excretion. In healthy persons isomers I and III represent approximately 25% and 75% of total urinary coproporphyrins, and 75% and 25% of total biliary coproporphyrins, respectively [96]. Koskelo et al. [97] were the first to report an abnormal urinary coproporphyrin excretion pattern in patients with Dubin-Johnson syndrome, an observation which has since been confirmed in other patients [98, 99]. In Dubin-Johnson syndrome the total urinary coproporphyrin excretion is unchanged, but more than 80% is coproporphyrin isomer type I [98–100]. The same phenomenon has been observed in the TR-/GY rat [11].

The mechanism of the abnormal urinary coproporphyrin excretion is not known, although a relationship with a defect in the biosynthesis of hepatic porphyrins was originally postulated [96, 98, 100]. A defective hepatic uroporphyrinogen III cosynthetase activity would account for a disturbed coproporphyrin type III synthesis and a subsequent shift to the formation of coproporphyrin type I, which might explain the increase of type I in urine. Shimizu et al. [101], however, demonstrated that this enzyme activity is not impaired in patients with Dubin-Johnson syndrome. A different explanation is based on transport studies in the TR-/GY rat, which identified coproporphyrin type I as an endogenous substrate for cMOAT [11]. Assuming that this transport activity is impaired in Dubin-Johnson syndrome, the hepatobiliary excretion of the type I isomer shifts towards the kidney, which results in increased urinary type I excretion. The

reduced urinary type III excretion might be explained by competition of type I and type III isomers for the same transport protein, in which the transport of type I isomers is favored. Furthermore, transport of total coproporphyrins might be competitively inhibited by bilirubin.

Obligate heterozygotes for Dubin-Johnson syndrome have a slightly increased isomer I/III ratio, which justifies the assumption that it is a recessive syndrome [99, 100]. In addition, the organic anion excretion defect was identified as an autosomal recessive characteristic of this syndrome in a study of BSP and bilirubin metabolism in obligate heterozygotes [102].

Conclusion

Since the above characteristics of Dubin-Johnson syndrome are highly similar to the phenotype of the TR-/GY rat, it is obvious to speculate that a mutation in the *cMOAT* gene underlies the transport defect in Dubin-Johnson syndrome. A recent report by Kartenbeck et al. [103] describes the absence of the cMRP/cMOAT protein in liver from a Dubin-Johnson patient. Using hMRP1-specific antibodies, they detected both lateral and canalicular staining in human normal control liver, while the canalicular staining is absent in a Dubin-Johnson liver. The homology between the hMRP1 protein and the cMRP/cMOAT protein (47% overall amino acid identity) probably causes cross-reaction of the antibodies between the two proteins, the hMRP1 protein being expressed in the lateral membrane [66], and the cMRP/cMOAT protein being expressed in the canalicular membrane of the hepatocyte [14, 74]. Recently the human *cMRP/cMOAT* cDNA has been isolated [74, 104, 105], and recent observations in our laboratory indicate that a mutated *cMOAT* gene accounts for the phenotype observed in patients with Dubin-Johnson syndrome [105]. Because Dubin-Johnson syndrome is a particularly rare and harmless disorder, it is questionable whether the isolation of the *cMOAT* gene will have any diagnostic value. In addition to this, it should be noted that the urinary coproporphyrin isomer I/III ratio has proven to be a reliable parameter in diagnosing Dubin-Johnson syndrome [106]. However, in cases of uncertainty cDNA sequencing may lead to a final diagnosis.

The identification of cMOAT contributes to our understanding of hepatic transport, and the process of bile formation which is valuable for the development of efficient drugs. Furthermore, it enables one to study the regulation of canalicular organic anion transport under several pathological conditions, including cholestasis and endotoxemia. The high homology and corresponding substrate specificity with hMRP1 raises the question of whether the cMOAT protein is an important determinant in the resistance of tumor cells against cytostatic drugs. This will have to be settled using *cMOAT*-transfected cells.

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