Multidrug resistance protein 2 (MRP2, ABCC2) is an efflux membrane transporter highly expressed in liver, kidney and intestine with important physiological and pharmacological roles. The goal of this study was to investigate the functional significance of promoter region polymorphisms in ABCC2 and potential allele-specific expression. Twelve polymorphisms in the 1.6 kb region upstream of the translation start site were identified by resequencing 247 DNA samples from ethnically diverse individuals. Luciferase reporter gene assays showed that $ABCC2$ –24C>T both alone and as part of a common haplotype ($–24C>T/–1019A>G$) increased promoter function 35% compared with the reference sequence ($P<0.0001$). No other common variants or haplotypes affected $ABCC2$ promoter activity. Allele-specific expression was also investigated as a mechanism to explain reported associations of the synonymous $ABCC2$ 3972C>T variant with pharmacokinetic phenotypes. In Caucasian liver samples ($n=41$) heterozygous for the 3972C>T polymorphism, the 3972C allele was preferentially transcribed relative to the 3972T allele ($P<0.0001$). This allelic imbalance was particularly apparent in samples with haplotypes containing two or three promoter/untranslated region variants ($–1549G>A$, –1019A>G and $–24C>T$). The observed allelic imbalance was not associated with hepatic or renal ABCC2 mRNA expression. Additional mechanisms will need to be explored to account for the interindividual variation in ABCC2 expression and MRP2 function.

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INTRODUCTION

The multidrug resistance protein 2 (MRP2), encoded by $ABCC2$, is a member of the ATP-binding cassette (ABC) family of membrane transporters. MRP2 is predominantly expressed in the canalicular membrane of hepatocytes, but can also be detected in the apical membranes of renal proximal tubule and intestinal epithelial cells. MRP2 has an important role in the pharmacokinetics of a wide variety of compounds. This transporter drives energy-dependent efflux of organic anions, often conjugated to glutathione, glucuronic acid or sulfate, into the bile or urine for elimination. It can also transport neutral or basic compounds, but requires glutathione for co-transport. MRP2 substrates include endogenous compounds such as glutathione, leukotriene $C_4$, 17β-estradiol glucuronide and the heme metabolite bilirubin glucuronide as well as xenobiotics such as the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitor pravastatin, and the anticancer drugs irinotecan and its glucuronide metabolite SN-38 glucuronide, cisplatin and methotrexate.

The importance of MRP2-mediated transport has been clearly demonstrated in mutant rat models lacking Mrp2 expression and in Dubin–Johnson syndrome patients with a hereditary deficiency of MRP2 caused by rare mutations in the coding region of $ABCC2$. Also, a number of common $ABCC2$ non-synonymous polymorphisms have been associated with alterations in ABCC2 mRNA expression and/or MRP2 function as well as with drug toxicities. The functional effects of $ABCC2$ coding region and promoter polymorphisms remain controversial. In particular, the mechanisms by which promoter and synonymous polymorphisms influence MRP2 expression are unclear. Currently, there is limited information about transcriptional regulation of human $ABCC2$. Several studies have characterized a region between 197 and 517 bases upstream from the translation start site with important basal promoter activity. This region contains consensus sequences for a number of transcription factors, including the TATA box, the liver abundant CCAAT-enhancer-binding protein and HNF1/4. Single-nucleotide polymorphisms (SNPs) in the promoter region may alter transcriptional activity and contribute to interindividual variability in MRP2 expression and the pharmacokinetics of MRP2 substrates.

To date, a number of SNPs have been associated with the pharmacokinetics, efficacy and toxicity of MRP2 substrates, although most findings have not been replicated. For example, the $ABCC2$ –24C>T polymorphism has been shown to decrease, increase as well as have no effect on ABCC2 expression. Interestingly, the $ABCC2$ synonymous variant 3972C>T, which is in linkage disequilibrium with a number of promoter region polymorphisms, was associated with exposure to irinotecan and its metabolite SN-38 glucuronide. Patients who were homozygous for the 3972T allele had higher area under the curves for irinotecan and SN-38 glucuronide compared with the combined group of patients having the CT or CC genotype.

Keywords: ABC transporter; MRP2; ABCC2; pharmacogenetics; promoter; allelic imbalance
The higher area under the curve is consistent with decreased activity/or expression of MRP2. In contrast, other studies have shown no effect of this polymorphism on ABCC2 expression and MRP2 function.9,10,27–29

This study represents a comprehensive functional analysis of genetic variants in the promoter region of ABCC2. Based on the reported associations of ABCC2 3972C > T with various phenotypes,22,30,31 allele-specific expression was also investigated as a possible mechanism conferring functional significance for this synonymous SNP.

MATERIALS AND METHODS

Tissue samples

Two hundred human liver samples were provided by the Liver Tissue Procurement and Distribution System NIH Contract #N01-DK-9-2310 and by the Cooperative Human Tissue Network and were processed through Dr Mary Relling’s laboratory at St Jude Children’s Research Hospital (Memphis, TN, USA); these liver samples were genotyped and used for ABCC2 allele-specific expression experiments. Additional kidney (n = 56) and liver (n = 34) samples were provided by the Pharmacogenomics of Membrane Transporters tissue repository at the University of California San Francisco and were used to correlate ABCC2 3972C > T genotype with total ABCC2 mRNA expression.

Genetic analysis of the 5′-region of ABCC2

A collection of 247 ethnically diverse genomic DNA samples were obtained from the Coriell Institute of Medical Research (http://coriell.org). This collection of samples was used to screen for genetic variations in a 1.6 kb upstream region of ABCC2. Initial screening was done using PCR, denaturing high-performance liquid chromatography and direct sequencing as previously described.52

Plasmid construction

A 1.6 kb region upstream of the ABCC2 translation start site was PCR amplified from genomic DNA and subcloned into pCR2.1 using the standard protocol from the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). The amplified promoter DNA was digested with Nhel and Xhol (New England Biolabs, Beverly, MA, USA) and was cloned into the reporter gene expression vector pGL4.11b (Promega, Madison, WI, USA), which contains a luciferase gene downstream from the multiple cloning site. Compared with the earlier generation pGL3 vectors, the pGL4 vectors are engineered with fewer consensus regulatory sequences and a synthetic gene, which has been codon optimized for mammalian expression. Individual non-singleton variants or variants in haplotypes were introduced using specific primers (Supplementary Table 1) and the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Plasmids were sequenced to verify the correct introduction of variants and were isolated as endotoxin-free preparations, a step that has been shown to eliminate purity bias and to significantly improve interassay variability.

Cell culture and reporter gene assay

HepG2 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. A day before transfection cells were counted and seeded on 96-well plates at a density of 5 × 10^4 cells per well. pGL4.11b empty vector or plasmids containing either reference or variant sequences were transiently transfected together with Renilla luciferase vector into HepG2 cells using the Lipofectamine 2000 reagent (Invitrogen). Renilla luciferase vector served as a transfection efficiency control. Cells were incubated for 24 h at 37 °C in humidified atmosphere with 5% CO₂, after which they were washed with phosphate-buffered saline and incubated with 80 μl of passive lysis buffer (Promega) at room temperature on a shaker for 30 min. Aliquots were analyzed for luciferase activity in a dual luciferase reporter assay on a GloMax 96 luminometer (Promega) according to the manufacturer’s instructions. The activities of Firefly and Renilla luciferases were measured sequentially from each sample and both reporters yield linear assays with subattomole (<10⁻¹⁸) sensitivities and no endogenous activity in the host cells.

Genotyping

Genomic DNA was genotyped using a 5′-nuclease assay33 and direct sequencing. The ABCC2 5′-promoter variants −1549G > A (rs1885301) and −1019A > G (rs2804402), the untranslated region (UTR) variant −24C > T (rs717620), the coding region variant 1249G > A (rs2273697), the intron 26 variant −34T > C (rs17216177) and the synonymous variant 3972C > T (rs3740066) were genotyped in 200 liver samples. Briefly, PCR for direct sequencing was carried out after optimization using a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Sequencing analysis was done on an ABI 3700 automated sequencer using the ABI PRISM BigDye system (Life Technologies Corporation, Carlsbad, CA, USA). For Taqman 5′-nuclease sequencing, PCR and sequencing were performed in one reaction using specific primers for the variant of interest, either by Assays-on-Demand (−24C > T and 3972C > T; Applied Biosystems) or Assays-by-Design (primer/probes in Supplementary Table 2). The ABCC2 3972C > T SNP was also genotyped in 56 liver and 34 kidney samples using the DMET™ array chip on an Illumina platform; the ABCC2 3972C > T SNP was tagged by rs7067971 SNP (r² = 1.0). In all genotyping assays, SNPs were checked for deviations from reported minor allele frequencies and also for Hardy–Weinberg equilibrium.

Reverse transcription, PCR and single base extension

RNA from human liver samples heterozygous for the ABCC2 3972C > T SNP were selected for reverse transcription and further analysis. One microgram of RNA was reverse transcribed using the SuperScript II Reverse Transcriptase kit (Invitrogen) according to the manufacturer’s protocol. Following reverse transcription, approximately 100 bp surrounding the 3972C > T SNP site was PCR amplified from the resulting complementary DNA and genomic DNA using specific primers. Excess dNTPs and primers were removed from the amplified product by incubating in shrimp alkaline phosphatase (Promega) and Exol (New England Biolabs). PCR products then underwent single base extension using the SNaPshot mix (Applied Biosystems) and extension primers. Allele abundance was measured by an ABI 3700 sequencer. Peak heights of each allele were recorded from the resulting chromatogram.

Allele-specific expression analysis

An allelic imbalance ratio was quantified by the following equation:

\[ R = \frac{C_1}{C_2} \frac{G_1}{G_2} \]

where \( C_1 \) and \( C_2 \) are the peak heights of one allele in the complementary DNA and genomic DNA, respectively, and \( G_1 \) and \( G_2 \) are the peak heights of the second allele. The genomic DNA ratio was used as an internal normalization control to account for any fluorophore differences between the two alleles. To equalize the ratios in graphical space, the values were log transformed. Log-transformed values were defined as follows: log \( R = 0 \), no preference for either allele; log \( R > 0 \), preference for allele 1; and log \( R < 0 \), preference for allele 2.

Quantifying mRNA expression in human liver and kidney samples

ABCC2 mRNA expression was measured in 56 Caucasian kidney and 34 Caucasian liver samples using BioTrove Open Array technology according to the manufacturer’s protocol (Life Technologies, Carlsbad, CA, USA). ABCC2 mRNA expression was normalized to a geometric mean of the expression of GAPDH, β-2 microglobulin, and β-actin and expressed as 2⁻^[ΔΔCt].

RESULTS

Polymorphisms in the proximal promoter region

To identify polymorphisms in the ABCC2 promoter region, a 1.6 kb region upstream from the ATG start site was sequenced from 247 ethnically diverse DNA samples from the Coriell Institute. Twelve...
polymorphisms were found in this region (Table 1), including two SNPs in the 5'-UTR (–1 to –247 bp from the translation start site). All other SNPs were found >700 bp upstream from the translation start site. An insertion was discovered at –1059 in a single chromosome in the African-American population. There were no variations found between –247 and –500 bp, the region required for basal expression.7 Four polymorphisms (rs1885301, rs7910642, rs2804402) were considered cosmopolitan and frequent and African-American specific. The rare and African-American specific haplotype containing the –733G > A SNP was specific to the Asian-American population, and the –23G > A UTR haplotype was specific to the African-American population. The –733G > A SNP was found at frequencies of 2%, 3% and 10%, respectively.

Table 1. Variants identified in the promoter region of ABCC2.

| SNP       | Positiona | Nucleotide change | Allele frequencyb |
|-----------|-----------|------------------|-------------------|
|           |           |                  | CA    | AA    | AS    | ME    | PA    |
| rs17222653 | –1563     | G→A              | 0.000 | 0.005 | 0.000 | 0.000 | 0.000 |
| rs1885301  | –1549     | G→A              | 0.430 | 0.485 | 0.150 | 0.200 | 0.357 |
| rs17222667 | –1292     | A→G              | 0.000 | 0.015 | 0.000 | 0.000 | 0.000 |
| rs17222646 | –1239     | G→A              | 0.000 | 0.005 | 0.000 | 0.050 | 0.000 |
| rs17216128 | –1065     | C→A              | 0.000 | 0.005 | 0.000 | 0.050 | 0.000 |
| rs45593436 | –1059     | insG             | 0.000 | 0.005 | 0.000 | 0.000 | 0.000 |
| rs7910642  | –1023     | G→A              | 0.150 | 0.135 | 0.267 | 0.300 | 0.429 |
| rs2804402  | –1019     | A→G              | 0.430 | 0.365 | 0.167 | 0.200 | 0.357 |
| rs17222533 | –798      | C→A              | 0.000 | 0.005 | 0.000 | 0.000 | 0.000 |
| rs17216135 | –733      | G→A              | 0.000 | 0.000 | 0.017 | 0.000 | 0.000 |
| rs717620   | –24       | C→T              | 0.195 | 0.060 | 0.150 | 0.150 | 0.286 |
| rs17216156 | –23       | G→A              | 0.000 | 0.000 | 0.000 | 0.000 | 0.143 |

Abbreviations: ABC,ATP-binding cassette; SNP, single-nucleotide polymorphism.

*Nucleotide positions are numbered based on the distance from the translation (+1) start site.

**Allele-specific expression**

Allele-specific expression was investigated as a possible mechanism for observed associations of ABCB2 regulatory polymorphisms with various phenotypes.22,30,31 Of 200 liver samples available, the majority of ABCC2 promoter variants on promoter activity in vitro To assess if promoter polymorphisms have an effect on ABC2 promoter activity, a 1.6 kb fragment was amplified from genomic DNA and cloned into the multiple cloning site of the promoterless luciferase plasmid, pGL4.11b. Single variant or promoter haplotypes were introduced by site-directed mutagenesis using specific primers. A plasmid containing the reference sequence showed approximately 70-fold higher activity than the promoterless pGL4.11b plasmid. The ABC2 –24C > T SNP, when introduced both as a single variant and as part of the –24C > T/–1019A > G/–1549G > A haplotype, showed a significant 35% increase in reporter activity compared with the reference ABC2 promoter sequence (P<0.0001). Reporter activity of promoter constructs carrying other SNPs and haplotypes were not significantly different from the reference (Figure 1). There was no association of the common ABC2 promoter variants (– 1549A > G, – 1023G > A and – 24C > T) with ABC2 mRNA levels in 34 Caucasian liver and 56 Caucasian kidney samples (data not shown).

Table 2. Ethnic distribution of ABCC2 promoter haplotypes.

| –1549G > A | –1292A > G | –1239G > A | –1023G > A | –1019A > G | –24C > T | Frequencyb |
|------------|------------|------------|------------|------------|----------|------------|
|            | CA         | AA         | AS         | ME         | PA       |            |
|            | G          | A          | G          | G          | A        | C          | 0.460 | 0.390 | 0.570 |
| A          | G          | C          | G          | A          | A        | C          | 0.120 | 0.260 | 0.000 |
| A          | G          | A          | G          | G          | G        | C          | 0.200 | 0.050 | 0.160 |
| A          | G          | G          | G          | G          | G        | C          | 0.000 | 0.030 | 0.000 |
| A          | G          | C          | A          | C          | A        | C          | 0.000 | 0.020 | 0.000 |
| A          | G          | C          | A          | C          | A        | C          | 0.000 | 0.100 | 0.000 |
| A          | G          | C          | A          | C          | A        | C          | 0.110 | 0.110 | 0.280 |

Abbreviations: ABC,ATP-binding cassette; SNP, single-nucleotide polymorphism.

*Promoter haplotypes were estimated for six non-singleton 5'-variants using PHASE and the haplotype frequencies are shown for the Caucasian (CA, n = 200), African American (AA, n = 200) and Asian American (AS, n = 60) populations. The first row is the promoter reference haplotype; nucleotide changes for each SNP site are indicated for the variant haplotypes.
(n = 137) were of known Caucasian descent and were used for this analysis. The sample sizes of other ethnic groups were too small for inclusion. Haplotypes were constructed using five additional polymorphisms commonly found with the 3972C>T variant and the distribution of these haplotypes in Caucasians is shown in Table 3. Ten different haplotypes were estimated for the 137 liver samples. A total of 53 Caucasian liver samples were heterozygous at the 3972C>T polymorphic site. Seven livers carried low-frequency haplotypes that were not considered further and five liver RNA samples could not be reverse transcribed and were also removed from the analysis. Results of the allele-specific expression analysis are presented for the most common haplotype pairs (n = 41; Figure 2).

Overall, there was a statistically significant preference for the 3972C allele compared with the 3972T allele (log ratios > 0, P < 0.0001). When examining haplotype pairs, H1/H2 (P < 0.01), H2/H3 (P < 0.0001) and H3/H4 (P < 0.01) showed a significant preference for the C allele, suggesting that H2 and H4 might be driving this allelic imbalance. Samples carrying H2/H3 showed the highest mean C/T ratios of all haplotype combinations; in these samples, the C allele was 14% higher in abundance than the T allele.

Association of hepatic and renal ABCC2 mRNA expression with 3972C>T genotype

To assess if 3972C>T allelic imbalance is pronounced enough to affect total ABCC2 mRNA expression, we genotyped 34 additional Caucasian liver and 56 Caucasian kidney samples for this SNP using a tagging SNP (rs7067971) and quantified ABCC2 mRNA expression. No association was observed in these samples between 3972C>T genotype and ABCC2 mRNA expression (Figure 3).

**DISCUSSION**

MRP2 is one of the most important efflux transporters and can transport a large number of drugs and their conjugates. The ABCC2 gene encoding MRP2 is highly polymorphic and genetic variability is associated with interindividual differences in pharmacokinetics, drug response and toxicity. In particular, non-synonymous coding region polymorphisms and disease mutations have been associated with changes in ABCC2 expression and/or MRP2 function.8–14 The effects of ABCC2 promoter and synonymous variants are less well understood. The purpose of this study was twofold; first, to identify and characterize the functional effect of common variants in the ABCC2 promoter and/or promoter region; second, to determine if allelic imbalance associated with the synonymous 3972C>T allele might provide mechanistic insight into the association of this variant with irinotecan disposition and toxicity.22,24,29,34

The proximal promoter region of ABCC2 has several common polymorphisms in the 1600 bp region upstream of the transcription start site. Interestingly, the region containing the core promoter (−300 to −500 bp)16,17 is devoid of any common genetic variation, consistent with a significant role in the constitutive regulation of ABCC2 expression. The most common promoter region haplotypes contain the −1549G>A and −1019G>A polymorphisms, often together with the −24C>T UTR variant. The association of −1549G>A and −1019G>A polymorphisms with increased irinotecan and/or SN-38 exposure following irinotecan treatment22,29,34 suggests that these promoter region variants might influence ABCC2 transcription and therefore expression.

However, our in vitro studies failed to show a significant effect of the −1549G>A and −1019G>A variants, alone or in combination, with in vitro promoter activity. Interestingly, the −24C>T variant,
alone and combined with the −1549G>A and −1019G>A variants, modestly increased promoter activity. If increased promoter activity translated into increased ABCC2 expression, then irinotecan and SN-38 exposure would be expected to be lower compared with patients with the reference sequence. However, ABCC2 −24C>T has been associated with increased irinotecan and SN-38 exposure, suggesting reduced transport of these substrates.22,29 Only limited studies have been previously carried out to characterize the functional impact of genetic polymorphisms in the ABCC2 promoter and 5′-UTR region. In cell-based reporter gene assays, the −24C>T/−1549G>A haplotype decreased promoter activity 39%20 and the −24C>T variant alone caused a 20% reduction in promoter activity.10 The reporter plasmids used in the current and previous studies contain different lengths of upstream DNA sequence, which may account for the varying results. In cell lines with different genotypes, the −24C>T polymorphism had no effect on DNA binding or mRNA stability but was associated with reduced protein expression and corresponding effects on transport function.35 Consistent with these cell-based findings, −24C>T had no effect on the expression of ABCC2 mRNA in hippocampal, intestinal, placental or adenocarcinoma samples.9,23,36–38 In contrast, lower levels of ABCC2 renal mRNA were reported in patients carrying the −24T allele.10 In addition to associations of −24C>T with irinotecan and SN-38 exposure, this variant has also been associated with increased diclofenac hepatotoxicity,21 greater risk of platinum hematological toxicity31 and antiepileptic drug resistance,36 and increased response to irinotecan and cisplatin treatment.24 Most of these findings have not been replicated, and in the case of antiepileptic drug resistance, recent data refutes the original association.39 A possible reason for the inconsistency between studies is that ABCC2 promoter SNPs previously associated with clinical phenotypes (for example, SN-38 pharmacokinetics and drug toxicities) are not causative and are in linkage disequilibrium with other SNPs (cis- or trans-acting) that are functional. In support of this alternative explanation, ENCODE data available on the UCSC genome browser indicate there are only two clusters of transcription factors binding in the ABCC2 promoter region and there is very little overlap of any SNPs previously associated with clinical phenotypes with this region. This publically available data are consistent with our findings that the commonly studied ABCC2 promoter SNPs are not critical for transcriptional regulation of ABCC2 and that other SNPs and transcriptional modulators may

Figure 2. Allele-specific expression in Caucasian livers. The log allelic imbalance ratio is plotted for 41 Caucasian livers. Each symbol represents an individual liver and the dashed line indicates a ratio where the C allele and T allele are equal in abundance. The mean values for each group are represented by lines. The allelic imbalance ratio is plotted for all samples and separately for each haplotype pair. Significant differences from 0 are noted (*P<0.01; **P<0.0001).

Figure 3. Association of ABCC2 3972C>T with ABCC2 mRNA expression. The relationship between ABCC2 3972 genotype and ABCC2 mRNA expression in kidney (a) and liver (b) samples is shown. ABCC2 mRNA expression is expressed relative to the geometric mean of the mRNA level of three housekeeping genes. Values are shown for individual samples and the mean values are indicated by the solid line.
be responsible for variability in MRP2 expression and function. For example, trans-acting SNPs as well as epigenetic mechanisms may be involved in the regulation of ABC2C2 expression. Further studies will be necessary to fully understand the functional and clinical impact of any of the ABC2C2 promoter/UTR polymorphisms.

**ABC2C2** –24C>T is in linkage disequilibrium with a synonymous 3972C>T polymorphism, raising the possibility that previously reported associations of the 5’-UTR variant with clinical phenotypes may reflect functional changes related to the synonymous variant. In allelic imbalance studies, we found a significant preference for the 3972C allele compared with the 3972T allele. This appears to be driven by a haplotype (H2) that includes polymorphisms at –1549, –1019 and –24 in ABC2C2, and H3-T includes only the –1549 and –1019 SNPs. The relative effect of the observed allelic imbalance is modest, with at most a 14% average increase in transcription of the 3972C allele compared with the T allele. However, a large degree of interindividual variability was observed in this measurement and up to 45% increases were measured. The allelic imbalance did not affect total hepatic or renal ABC2C2 mRNA levels, suggesting its clinical significance will be limited except perhaps in individuals with extreme degrees of allele-specific expression.

A recent paper suggests that the 3972C>T variant could also be acting through a post-transcriptional mechanism. The –24C>T variant was associated with a modest decrease in MRP2 recombini-ant protein expression, while 3972C>T variant had much more profound effect and drove the observed decrease in protein expression with a 247/3972T haplotype. Interestingly, no effect on mRNA expression was observed, suggesting these polymorphisms have an important role in MRP2 translational regulation. The contribution of genetic variation to post-transcriptional regulation of ABC2C2/MRP2 expression should be further explored. Additional mechanisms for regulation of ABC2C2/MRP2, including expression quantitative trait loci and epigenetics, also warrant investigation. Interindividual variation in these mechanisms may contribute to variation in transport of endogenous and xenobiotic substrates of MRP2.

In summary, a number of common promoter and 5’-UTR polymorphisms in ABC2C2 are found at relatively high frequency across different ethnic groups. The limited, if any, effect of these variants on promoter activity suggests that reported associations with clinical phenotypes are not a result of impaired transcription. The 3972C>T synonymous variant is associated with allelic-specific expression, although the magnitude of the effect is modest. Additional mechanisms will need to be explored to account for the reported association of ABC2C2 promoter variants with irinotecan disposition and other drug response phenotypes.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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**REFERENCES**

1 Paulusma CC, Oude Elferink RP. The canalicular multispecific organic anion transporter and conjugated hyperbilirubinemia in rat and man. J Mol Med 1997; 75: 420–428.

2 Buchler M, Konig J, Brom M, Kartenbeck J, Spring H, Horie Y et al. cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. J Biol Chem 1996; 271: 15091–15098.

3 Dietrich CG, Ottenhoff R, de Waart DR, Oude Elferink RP. Role of MRP2 and GSH in intrahepatic cycling of toxins. Toxicology 2001; 167: 73–81.

4 Evers R, de Haas M, Spardians R, Beijnen J, Wielinga PR, Lankelma J et al. Vinblastine and sulfipyrazone export by the multidrug resistance protein MRPI is associated with glutathione export. Br J Cancer 2000; 83: 375–383.

5 Nies AT, Keppler D. The apical conjugate efflux pump MRP2 (ABCC2). Pflugers Arch 2007; 455: 643–659.

6 Jennitz K, Heredi-Szabo K, Janossy J, Iloa E, Verekzyk L, Krajczi P. ABC2C2/Abcc2: a multidrug transporters with dominant excretory functions. Drug Metab Rev 2010; 42: 402–436.

7 Toh S, Wada M, Uchiumi T, Inokuchi A, Makino Y, Horie Y et al. Genomic structure of the canalicular multispecific organic anion transporter gene (MRP2/(ABCC2)) and mutations in the ATP-binding-cassette region in Dublin-Johnson syndrome. Am J Hum Genet 1999; 64: 739–746.

8 Anderson PL, Lam J, Aquilante CL, Schuetz E, Fletcher CV. Pharmacogenetic characteristics of indinavir, zidovudine, and lamivudine therapy in HIV-infected adults: a pilot study. J Acquir Immune Defic Syndr 2006; 42: 441–449.

9 Haerisch S, May K, Wegner D, Calebe A, Cascorbi I, Siegmund W. Influence of genetic polymorphisms on intestinal expression and rifampicin-type induction of ABCC2 and on bioavailability of talinolol. Pharmacogenet Genomics 2008; 18: 357–365.

10 Haenisch S, Zimmermann U, Dazert E, Wruck CJ, Dazert P, Siegmund W et al. Influence of polymorphisms of ABCB1 and ABCG2 on mRNA and protein expression in normal and cancerous kidney cortex. Pharmacogenomics J 2007; 7: 56–65.

11 Hirouchi M, Suzuki H, Itoda M, Ozawa S, Sawada J, Ieiri I et al. Characterization of the cellular localization, expression level, and function of SNP variants of MRPI/ ABC2C2. Pharm Res 2004; 21: 742–748.

12 Meier Y, Paul-Magnus C, Zanger UM, Klein K, Schaeffeler E, Nussler AK et al. Interindividual variability of canalicular ATP-binding-cassette (ABC)-transporter expression in human liver. Hepatology 2006; 44: 62–74.

13 Ranganathan P, Culverhouse R, Marsh S, Mody A, Scott-Horton TJ, Brasington R et al. Methotrexate (MTX) pathway gene polymorphisms and their effects on MTX toxicity in Caucasian and African American patients with rheumatoid arthritis. J Rheumatol 2005; 32: 572–579.

14 Izdeine H, Hulot JS, Villard E, Goyenvalle C, Dominguez S, Ghosn J et al. Association between ABC2C2 gene haplotypes and tenofovir-induced proximal tubulopathy. J Infect Dis 2006; 194: 1481–1491.

15 Wojnowski L, Kulle B, Schirmer M, Schluter G, Schmidt A, Rosenberger A et al. NADPH oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. Circulation 2005; 112: 3754–3762.

16 Stockel B, Konig J, Nies AT, Cui Y, Brom M, Keppler D. Characterization of the 5’-flanking region of the human multidrug resistance protein 2 (MRP2) gene and its regulation in comparison with the multidrug resistance protein 3 (MRP3) gene. Eur J Biochem 2000; 267: 1347–1358.

17 Tanaka T, Uchiumi T, Hinoshita E, Inokuchi A, Toh S, Wada M et al. The human multidrug resistance protein 2 gene: functional characterization of the 5’-flanking region and expression in hepatic cells. Hepatology 1999; 30: 1507–1512.

18 Qadri I, Hu L, Iwahashi M, Al-Zubi S, Quattrochi LC, Simon FR. Interaction of hepatocyte nuclear factors in transcriptional regulation of tissue specific hormonal expression of human multidrug resistance-associated protein 2 (ABCG2). Toxicol Appl Pharmacol 2009; 234: 281–292.

19 Naesens M, Kuypers DR, Verbeke K, Vanrenterghem Y. Multidrug resistance protein 2 genetic polymorphisms influence mycobacterial acid secretion in renal allograft recipients. Transplantation 2006; 82: 1074–1084.

20 Choi JH, Ahn BM, Yi J, Lee JH, Nam SW, Chun CY et al. MRP2 haplotypes confer differential susceptibility to toxic liver injury. Pharmacogenet Genomics 2007; 17: 403–415.

21 Daly AK, Atiahl GP, Leahant HB, Swainson RA, Dang TS, Day CP. Genetic susceptibility to diclofenac-induced hepatotoxicity: contribution of UGT2B7, CYP2C8, and ABC2C2 genotypes. Gastroenterology 2007; 132: 272–281.

22 Fujita K, Nagashima F, Yamamoto W, Endo H, Sunakawa Y, Yamashita K et al. Association of ATP-binding cassette, sub-family C, number 2 (ABCC2) genotype with pharmacokinetics of irinotecan in Japanese patients with metastatic colorectal cancer treated with irinotecan plus infusional 5-fluorouracil/leucovorin (FOLFIRI). Biol Pharm Bull 2008; 31: 2137–2142.

23 Moriya Y, Nakamura T, Horinouchi M, Sakaeda T, Tamura T, Aoyama N et al. Effects of polymorphisms of MDR1, MRPI, and MRP2 genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects. Biol Pharm Bull 2002; 25: 1356–1359.

24 Han JY, Lim HS, Yoo YK, Shin ES, Park YH, Lee SY et al. Associations of ABCB1, ABC2C2, and ABC2C2 polymorphisms with irinotecan-pharmacokinetics and clinical outcome in patients with advanced non-small cell lung cancer. Cancer 2007; 110: 138–147.

25 Miura M, Satoh S, Inoue K, Kagaya H, Saito M, Inoue T et al. Influence of SLCO1B1, 183, 281 and ABC2C2 genetic polymorphisms on mycopHENolic acid pharmacokinetics in Japanese renal transplant recipients. Eur J Clin Pharmacol 2007; 63: 1161–1169.
26 Innocenti F, Ratain MJ. Pharmacogenetics of irinotecan: clinical perspectives on the utility of genotyping. Pharmacogenomics 2006; 7: 1211–1221.
27 Kroetz DL. Role for drug transporters beyond tumor resistance: hepatic functional imaging and genotyping of multidrug resistance transporters for the prediction of irinotecan toxicity. J Clin Oncol 2006; 24: 4225–4227.
28 Zamboni WC, Ramanathan RK, McLeod HL, Mani S, Potter DM, Strychor S et al. Disposition of 9-nitrocamptothecin and its 9-aminocamptothecin metabolite in relation to ABC transporter genotypes. Invest New Drugs 2006; 24: 393–401.
29 Innocenti F, Kroetz DL, Schuetz E, Dolan ME, Ramirez J, Relling M et al. Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. J Clin Oncol 2009; 27: 2604–2614.
30 Hobbinger A, Grunhage F, Sauerbruch T, Lammert F. Association of the c.3972C>T variant of the multidrug resistance-associated protein 2 Gene (MRP2/ABCC2) with susceptibility to bile duct cancer. Digestion 2009; 80: 36–39.
31 Han B, Gao G, Wu W, Gao Z, Zhao X, Li L et al. Association of ABC2 polymorphisms with platinum-based chemotherapy response and severe toxicity in non-small cell lung cancer patients. Lung Cancer 2011; 72: 238–243.
32 Leabman MK, Huang CC, DeYoung J, Carlson EJ, Taylor TR, de la Cruz M et al. Natural variation in human membrane transporter genes reveals evolutionary and functional constraints. Proc Natl Acad Sci USA 2003; 100: 5896–5901.
33 Latif S, Bauer-Sardina I, Ranade K, Livak KJ, Kwok PY. Fluorescence polarization in homogeneous nucleic acid analysis II: 5’-nuclease assay. Genome Res 2001; 11: 436–440.
34 de Jong FA, Scott-Horton TJ, Kroetz DL, McLeod HL, Friberg LE, Mathijssen RH et al. Irinotecan-induced diarrhea: functional significance of the polymorphic ABC2 transporter protein. Clin Pharmacol Ther 2007; 81: 42–49.
35 Laechelt S, Turrini E, Ruehmkorf A, Siegmund W, Casaroli I, Haensch S. Impact of ABC2 haplotypes on transcriptional and posttranscriptional gene regulation and function. Pharmacogenomics J 2011; 11: 25–34.
36 Ufer M, Mosyagin I, Muhle H, Jacobsen T, Haensch S, Hasler R et al. Non-response to antiepileptic pharmacotherapy is associated with the ABC2 —24C>T polymorphism in young and adult patients with epilepsy. Pharmacogenet Genomics 2009; 19: 353–362.
37 Nishioka C, Sakaeda T, Nakamura T, Moriya Y, Okamura N, Tamura T et al. MDR1, MRPI and MRP2 genotypes and in vitro chemosensitivity in Japanese patients with colorectal adenocarcinomas. Kobe J Med Sci 2004; 50: 181–188.
38 Meyer zu Schwabedissen HE, Jedlitschky G, Gratz M, Haensch S, Linnemann K, Fusch C et al. Variable expression of MRPI (ABCC2) in human placenta: influence of gestational age and cellular differentiation. Drug Metab Dispos 2005; 33: 896–904.
39 Hilger E, Reinthaler EM, Stogmann E, Hotzy C, Pataraia E, Baumgartner C et al. Lack of association between ABC2 gene variants and treatment response in epilepsy. Pharmacogenomics 2012; 13: 185–190.

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