



Published in final edited form as:

*Pharmacogenomics J.* 2013 October ; 13(5): 396–402. doi:10.1038/tpj.2012.20.

## FUNCTIONAL CHARACTERIZATION OF *ABCC2* PROMOTER POLYMORPHISMS AND ALLELE SPECIFIC EXPRESSION

Tan D. Nguyen, Ph.D.<sup>\*</sup>, Svetlana Markova, Ph.D.<sup>\*</sup>, Wanqing Liu, Ph.D., Jason M. Gow, Ph.D., R. Michael Baldwin, Ph.D., Mahnoush Habashian, B.S., Mary V. Relling, Pharm.D., Mark J. Ratain, M.D., and Deanna L. Kroetz, Ph.D.

Department of Bioengineering and Therapeutic Sciences, University of California San Francisco (TDN, SM, JMG, RMB, MH and DLK), Institute for Human Genetics, University of California San Francisco (DLK), Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital (MVR), and Department of Medicine, University of Chicago (WL and MJR)

### Abstract

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/-1549G>A) increased promoter function 35% compared to 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/UTR 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.

### Keywords

ABC transporter; MRP2; *ABCC2*; pharmacogenetics; promoter; allelic imbalance

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Corresponding Author: Deanna L. Kroetz, Ph.D., Department of Bioengineering and Therapeutic Sciences, Rock Hall 584E, Box 2911, San Francisco, CA 94158-2911, [deanna.kroetz@ucsf.edu](mailto:deanna.kroetz@ucsf.edu), Phone: 415-476-1159, Fax: 415-514-4361.

<sup>\*</sup>These authors contributed equally to this work

### CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## 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.<sup>1, 2</sup> MRP2 plays 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 (GSH), glucuronic acid or sulfate, into the bile or urine for elimination. It can also transport neutral or basic compounds, but requires GSH for co-transport.<sup>3, 4</sup> MRP2 substrates include endogenous compounds such as GSH, leukotriene C<sub>4</sub>, 17β-D-estradiol glucuronide and the heme metabolite bilirubin glucuronide as well as xenobiotics such as the HMG-CoA reductase inhibitor pravastatin, and the anti-cancer drugs irinotecan and its glucuronide metabolite SN-38 glucuronide, cisplatin and methotrexate.<sup>5, 6</sup>

The importance of MRP2 mediated transport has been clearly demonstrated in mutant rat models lacking *Mrp2* expression<sup>1</sup> and in Dubin-Johnson syndrome patients with a hereditary deficiency of MRP2 caused by rare mutations in the coding region of *ABCC2*.<sup>5-7</sup> Also, a number of common *ABCC2* non-synonymous polymorphisms have been associated with alterations in *ABCC2* mRNA expression and/or MRP2 function<sup>8-12</sup> as well as with drug toxicities.<sup>13-15</sup>

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.<sup>16-18</sup> 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<sup>19-22</sup>. For example, the *ABCC2* -24C>T polymorphism has been shown to decrease,<sup>10, 19-21</sup> increase<sup>8</sup> as well as have no effect on *ABCC2* expression.<sup>23-25</sup> 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.<sup>22, 26</sup> Patients who were homozygous for the 3972T allele had higher AUCs for irinotecan and SN-38 glucuronide compared to the combined group of patients having the CT or CC genotype. The higher AUC is consistent with decreased activity and/or expression of MRP2. In contrast, other studies have shown no effect of this polymorphism on *ABCC2* expression and MRP2 function.<sup>9, 10, 27-29</sup>

The current 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<sup>22, 30, 31</sup>, 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); 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 Pharmacogenetics 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.umdnj.edu>). 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 HPLC and direct sequencing as previously described.<sup>32</sup>

### 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). The amplified promoter DNA was digested with *NheI* and *XhoI* (New England Biolabs, Beverly, MA) and was cloned into the reporter gene expression vector pGL4.11b (Promega, Madison, WI), which contains a luciferase gene downstream from the multiple cloning site. Compared to 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 (Supplemental Table 1) and the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). 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 (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). A day prior to transfection cells were counted and seeded on 96 well plates at a density of  $5 \times 10^4$  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, Carlsbad, CA). *Renilla* luciferase vector served as a transfection efficiency control. Cells were incubated for 24 hours at 37°C in humidified atmosphere with 5% CO<sub>2</sub>, after which they were washed with phosphate buffered saline and incubated with 80 µL of passive lysis buffer (Promega, Madison, WI) 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, Madison, WI) 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<sup>-18</sup>) sensitivities and no endogenous activity in the host cells.

### Genotyping

Genomic DNA was genotyped using a 5'-nuclease assay<sup>33</sup> and direct sequencing. The *ABCC2* 5'-promoter variants -1549G>A (rs1885301) and -1019A>G (rs2804402), the 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). Sequencing analysis was done on an ABI 3700 automated sequencer using the ABI PRISM BigDye system. 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, Foster City, CA) or Assays-by-Design (primer/probes in Supplemental 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 ( $r^2 = 1.0$ ). 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, Carlsbad, CA) according to the manufacturer's protocol. Following reverse transcription, approximately 100 bp surrounding the 3972C>T SNP site was PCR amplified from the resulting cDNA and genomic DNA (gDNA) using specific primers. Excess dNTPs and primers were removed from the amplified product by incubating in shrimp alkaline phosphatase (Promega, Madison, WI) and *ExoI* (New England Biolabs, Beverly, MA). PCR products then underwent single base extension using the SNaPshot mix (Applied Biosystems, Foster City, CA) 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  $G_1$  are the peak heights of one allele in the cDNA and gDNA, respectively, and  $C_2$  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). ABCC2 mRNA expression was normalized to a geometric mean of the expression of GAPDH, beta-2 microglobulin, and beta-actin and expressed as  $2^{-C_t}$ .

## 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'-untranslated region (-1 to -247 bp from the translation start site). All other SNPs were found more than 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 and rs717620) were considered cosmopolitan and were found at greater than 5% frequency in each ethnic population. The -798C>A, -1059+G, -1292A>G and -1563G>A were rare and African American specific. The -733G>A variant was specific to the Asian American population, and the -23G>A UTR SNP was Pacific Islander specific. The non-singleton variants in the Caucasian, African American and Asian American populations were organized into eight different promoter haplotypes (Table 2). In addition to the reference haplotype, a haplotype containing the -1549G>A, -1019A>G and -24C>T variants and a second containing only the -1023G>A variant were found at high frequencies in each of the ethnic groups. The combination of the -1549G>A and -1019A>G variants was common in Caucasians and African Americans (frequency of 12% and 26%, respectively) but not observed in the Asian American population. Three African American specific haplotypes were identified, specifically -1549G>A/-1292A>G/-1019A>G, 1549G>A/-1239G>A/-1019A>G and -1549G>A, at frequencies of 2%, 3% and 10%, respectively.

### Effects of ABCC2 Promoter Variants on Promoter Activity In Vitro

To assess if promoter polymorphisms have an effect on *ABCC2* promoter activity a 1.6 kb fragment was amplified from genomic DNA and cloned into the multiple cloning site of the promoter-less 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 promoter-less pGL4.11b plasmid. The *ABCC2* -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 to the reference *ABCC2* 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 *ABCC2* promoter variants (-1549A>G, -1023G>A and -24C>T) with *ABCC2* mRNA levels in 34 Caucasian liver and 56 Caucasian kidney samples (data not shown).

### Allele Specific Expression

Allele specific expression was investigated as a possible mechanism for observed associations of *ABCC2* 3972C>T with various phenotypes.<sup>22, 30, 31</sup> Of 200 liver samples available, the majority (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 to 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.<sup>8–14</sup> The effects of *ABCC2* promoter and synonymous variants are less well understood. The purpose of this study was two-fold; first, to identify and characterize the functional effect of common variants in the *ABCC2* promoter and 5'-UTR. In addition, studies were carried out 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.<sup>22, 24, 29, 34</sup>

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<sup>16, 17</sup>) 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, -1019G>A and -24C>T polymorphisms with increased irinotecan and/or SN-38 exposure following irinotecan treatment<sup>22, 29, 34</sup> 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 to 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.<sup>22, 29</sup>

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%<sup>20</sup> and the -24C>T variant alone caused a 20% reduction in promoter activity.<sup>10</sup> 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.<sup>35</sup> Consistent with these cell-based findings, -24C>T had no effect on the expression of *ABCC2* mRNA in hippocampal, intestinal, placental or adenocarcinoma samples.<sup>9, 23, 36–38</sup> In contrast, lower levels of *ABCC2* renal mRNA were reported in patients carrying the -24T allele.<sup>10</sup> In addition to associations of -24C>T with irinotecan and SN-38 exposure, this variant has also been associated with increased diclofenac hepatotoxicity<sup>21</sup>, greater risk of platinum hematological toxicity<sup>31</sup> and antiepileptic drug resistance<sup>36</sup>, and increased response to irinotecan and cisplatin treatment.<sup>24</sup> Most of these findings have not been replicated, and in the case of antiepileptic drug resistance, recent data refutes the original association.<sup>39</sup> A possible reason for the inconsistency between studies is that *ABCC2* promoter SNPs

previously associated with clinical phenotypes (*e.g.*, 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 indicates 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 is 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 be responsible for variability in *MRP2* expression and function. For example, transacting SNPs as well as epigenetic mechanisms may be involved in the regulation of *ABCC2* expression. Further studies will be necessary to fully understand the functional and clinical impact of any of the *ABCC2* promoter/UTR polymorphisms.

*ABCC2* -24C>T is in linkage disequilibrium (LD) 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 to the 3972T allele. This appears to be driven by a haplotype (H2) that includes polymorphisms at -1549, -1019 and -24 in *ABCC2*, and H4 which 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 to 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 *ABCC2* 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 posttranscriptional mechanism. The -24C>T variant was associated with a modest decrease in *MRP2* recombinant protein expression, while 3972C>T variant had much more profound effect and drove the observed decrease in protein expression with the -24T/3972T haplotype.<sup>35</sup> Interestingly, no effect on mRNA expression was observed, suggesting these polymorphisms play an important role in *MRP2* translational regulation. The contribution of genetic variation to posttranscriptional regulation of *ABCC2*/*MRP2* expression should be further explored. Additional mechanisms for regulation of *ABCC2*/*MRP2*, including eQTLs 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 *ABCC2* 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 *ABCC2* promoter variants with irinotecan disposition and other drug response phenotypes.



## Acknowledgments

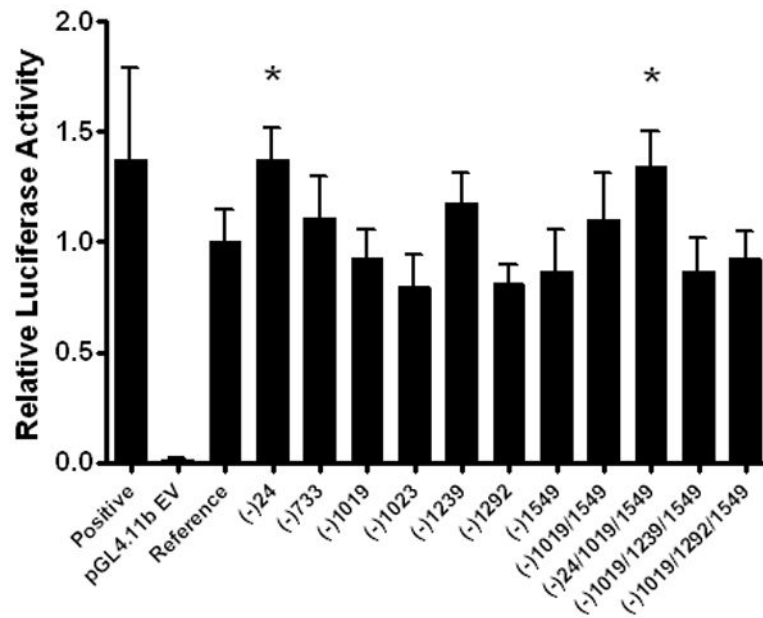
This work was supported by NIH grants GM61390, GM61393 and CA21765. Tan D. Nguyen and Jason M. Gow were supported in part by NIH Training Grant T32 GM007175.

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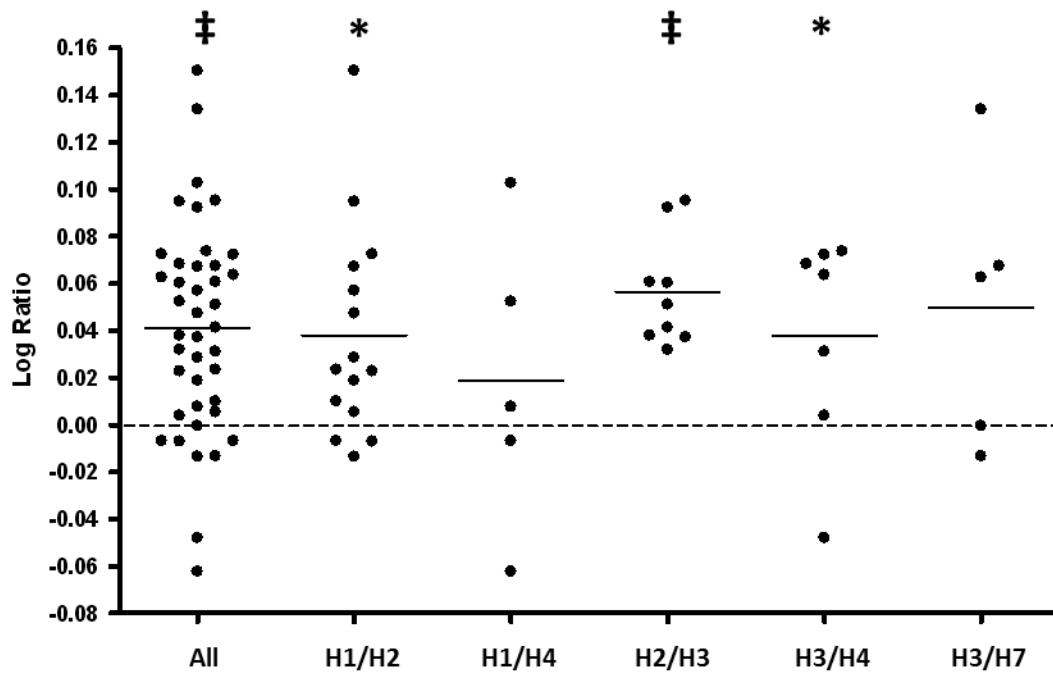
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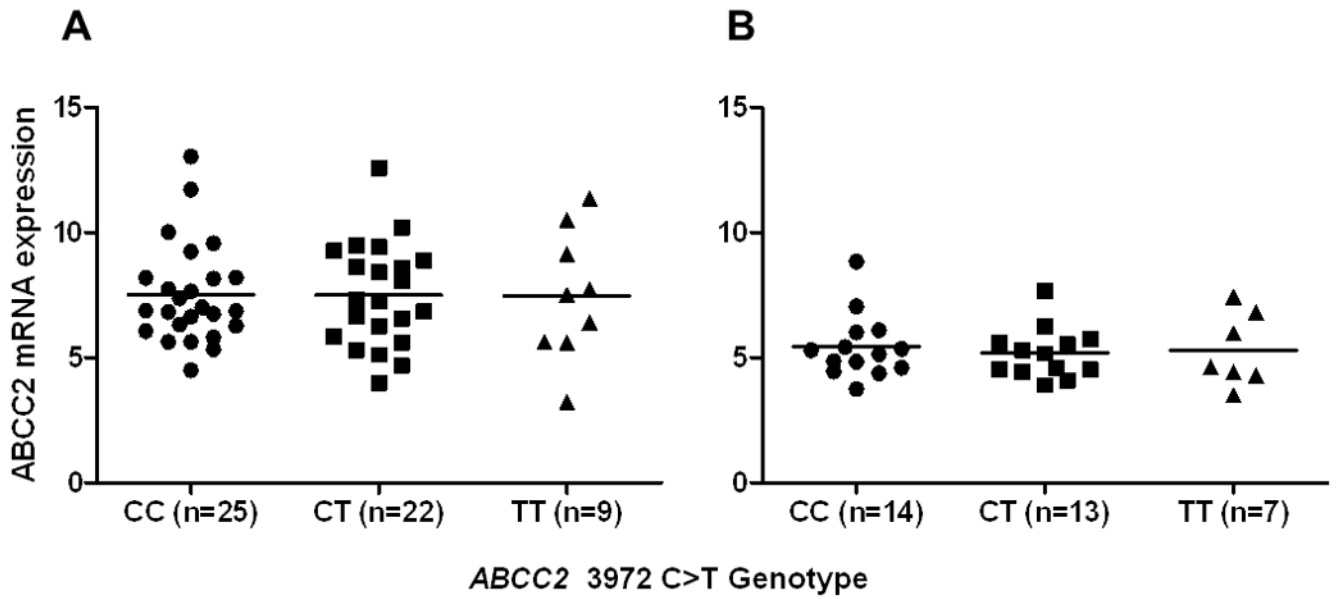
**Figure 1. Functional activity of *ABCC2* promoter variants**

*ABCC2* promoter activity was expressed as *Firefly* luciferase activity relative to *Renilla* luciferase activity; the activity of the reference sequence was set to one. Each column represents the mean ± SD of four separate transfection experiments performed in triplicate. The positive control was a liver enhancer sequence. \* $P < 0.001$ , significantly different from reference.



**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.



**Table 1**

Variants Identified in the Promoter Region of *ABCC2*

SNP	Position <sup>1</sup>	Nucleotide Change	Allele Frequency <sup>2</sup>			
			CA	AA	AS	PA
rs17222653	-1563	G>A	0.000	0.005	0.000	0.000
rs1885301	-1549	G>A	0.430	0.485	0.150	0.357
rs17222667	-1292	A>G	0.000	0.015	0.000	0.000
rs17222646	-1239	G>A	0.005	0.030	0.000	0.050
rs17216128	-1065	C>A	0.000	0.005	0.000	0.000
rs45593436	-1059	insG	0.000	0.005	0.000	0.000
rs7910642	-1023	G>A	0.150	0.135	0.267	0.300
rs2804402	-1019	A>G	0.430	0.365	0.167	0.200
rs17222533	-798	C>A	0.000	0.005	0.000	0.000
rs17216135	-733	G>A	0.000	0.000	0.017	0.000
rs717620	-24	C>T	0.195	0.060	0.150	0.286
rs17216156	-23	G>A	0.000	0.000	0.000	0.143

<sup>1</sup> Nucleotide positions are numbered based on the distance from the translation (+1) start site.

<sup>2</sup> Frequencies were calculated for each ethnic group: CA, Caucasians (n=200); AA, African Americans (n=200); AS, Asian Americans (n=60); ME, Mexican Americans (n=20); PA, Pacific Islanders (n=14).

**Table 2**

Ethnic Distribution of *ABCC2* Promoter Haplotypes

	Frequency <sup>I</sup>							
	-1549G>A	-1292A>G	-1239G>A	-1023G>A	-1019A>G	-24C>T	CA	AA
G	A	G	G	A	C	0.460	0.390	0.570
A			G	G		0.120	0.260	0.000
A				G	T	0.200	0.050	0.160
A		A		G		0.000	0.030	0.000
A	G			G		0.000	0.020	0.000
A			A			0.000	0.100	0.000
						0.110	0.110	0.280

<sup>I</sup> 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.

**Table 3**

Inferred *ABCC2* Haplotypes of Caucasian Liver Samples

	-1549G>A	-1019A>G	-24C>T	1249G>A	Intron 26-34T>C	3972C>T	Frequency <sup>I</sup>
<b>H1</b>	G	A	C	G	T	C	0.310
<b>H2</b>	A	G	T			T	0.234
<b>H3</b>				A			0.193
<b>H4</b>	A	G				T	0.117
<b>H5</b>	A	G					0.058
<b>H6</b>	A	G		C			0.033
<b>H7</b>						T	0.036
<b>H8</b>					C		0.011
<b>H9</b>	A	G	T				0.004
<b>H10</b>	A						0.000
<b>H11</b>				A		T	0.004
<b>H12</b>	A				C		0.000

<sup>I</sup> Promoter haplotypes in 137 Caucasian liver samples were estimated for six variants using PHASE and the haplotype frequencies are shown. H1 is the promoter reference haplotype; nucleotide changes for each SNP site are indicated for the variant haplotypes.