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Application of multivariate statistical procedures to identify transcription factors that correlate with MRP2, 3, and 4 mRNA in adult human livers

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Abstract

1. Multidrug resistance-associated proteins 2-4 (MRP2-4) are membrane efflux transporters that are critical for the hepatic clearance of pharmaceuticals and endogenous chemicals. Little is known about the constitutive regulation of MRP2-4 mRNA in normal human liver.
2. The purpose of this study was to identify transcription factors whose expression significantly correlates with MRP2-4 mRNA in human liver specimens.
3. Ninety adult human livers were profiled for mRNA expression of MRP2-4 as well as AhR, CAR, PXR, PPAR α and γ , LXR α , FXR, GR, RXR α , HNF1 α and 4 α , and Nrf2 transcription factors. Using linear regression and stepwise selection of partial R² values, CAR, HNF1 α , and PPAR α mRNA exhibited the greatest correlation with MRP2, 3, and 4, respectively.
4. Interindividual variation in the expression of the identified transcription factors may account for the variability in constitutive mRNA levels of MRP2-4. The multivariate approach presented in this study should aid in predicting signaling pathways that participate either directly or indirectly in regulating hepatic drug disposition.

Keywords

ABCC; Transporters; CAR; PPARalpha; HNF-1alpha

Introduction

The safety and efficacy of pharmacotherapy regimens are influenced by interindividual variability in pharmacokinetics and pharmacodynamics. This variance is governed by many factors including gender, age, ethnicity, concomitant medications, environmental exposures, underlying disease states, and polymorphisms. However, there are likely additional determinants that dictate the constitutive expression of genes involved in hepatic drug clearance that have yet to be elucidated. One potential determinant may be the expression levels of transcription factors that regulate metabolism and transport genes.

The hepatic clearance of drugs involves a series of events including uptake of chemicals into the liver by passive diffusion or carrier-mediated processes. Subsequently, drugs are metabolized by Phase-I and -II enzymes to produce water-soluble forms that are readily

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excreted by ATP-dependent membrane transporters. The primary hepatic efflux transporters are the multidrug resistance-associated proteins (MRP, human; Mrp, rodent) that belong to the ATP-binding cassette, subfamily c gene family. MRP3 and 4 localize to the sinusoidal membrane and excrete substrates from the hepatocyte to the blood. In contrast, canalicular MRP2 transports chemicals into bile. MRP2-4 isoforms exhibit differences in substrate preference. For example, Mrp2/MRP2 prefers to excrete glutathione conjugates whereas Mrp3/MRP3 predominantly transports glucuronidated chemicals (Manautou et al. 2005; Zelcer et al. 2005; Lagas et al. 2006; Vlaming et al. 2006; Zamek-Gliszczynski et al. 2006; van de Wetering et al. 2007). Sulfated xenobiotics are good substrates for Mrp4/MRP4-mediated transport (Mennone et al. 2006; Zamek-Gliszczynski et al. 2006). Comparison of nucleotide sequences using the NIH BLAST2 algorithm revealed 81-89% similarity between mouse and human *MRP2-4* genes, indicating potential overlap in substrate specificity between these two species (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/>). The recent development of knockout mice will enable further analysis of the *in vivo* roles of Mrp2-4 in hepatic drug clearance.

Both *in vivo* rodent studies and *in vitro* research with primary human hepatocytes demonstrate the basal and inducible regulation of transporters by ligand-activated transcription factors (Wang and LeCluyse 2003; Klaassen and Slitt 2005). Transcription factors such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator activated-receptor (PPAR) have documented roles in regulating Mrp expression and are expressed in liver (Klaassen and Slitt 2005). Human PXR has also been termed the steroid and xenobiotic receptor. The glucocorticoid receptor (GR) is an additional member of the nuclear hormone receptor family, although its ability to regulate Mrp transporters is unknown. A number of bile acid, sterol, and oxysterol sensors are also expressed in liver. Liver X receptor (LXR) and the farnesoid X receptor (FXR) may be relevant to the current study because MRP3 and MRP4 can transport bile acids into plasma and likely represent alternate bile acid transport pathways during cholestasis (Slitt et al. 2007). With the exception of GR, the receptors listed above heterodimerize with retinoid X receptor alpha (RXR α) and bind to responsive elements in the upstream regions of target genes and activate target gene transcription.

The liver also expresses other transcription factors that may control expression of nuclear hormone receptors and/or transporters. These include the hepatocyte nuclear factors (HNF) 1 α and 4 α (Cereghini 1996; Schrem et al. 2002). It was recently shown that HNF4 α may function as a master regulator dictating expression of metabolism and transporter genes as well as nuclear hormone receptors (Odom et al. 2004; Wortham et al. 2007). Finally, the transcription factor nuclear factor E2-related factor 2 (Nrf2) regulates mouse Mrp2-4 expression during periods of glutathione depletion, chemical induction, and acetaminophen-induced hepatotoxicity (Maher et al. 2005; 2007; Aleksunes et al. 2008). Nrf2 is activated in liver by electrophiles and/or oxidative stress to coordinately up-regulate cytoprotective genes. Collectively, these transcription factors participate in regulating Mrp expression in rodent models and *in vitro* hepatocyte systems via chemical induction and/or hepatotoxicant exposure.

There is limited information regarding the constitutive regulation of MRP mRNA expression in human liver. A recent report by Wortham *et al.* used 20 human livers and observed a relationship between MRP2 and HNF4 α mRNA expression (Wortham et al. 2007). The purpose of the current study is to identify the influence of variation in mRNA expression of transcription factors on constitutive levels of hepatic MRP2-4 mRNA in 90 adult human liver specimens. The multivariate statistical analyses employed in the present study identify transcription factors whose mRNA expression exhibits the highest association with MRP

expression. Additional analysis examines potential interrelationships between receptors and transcription factors that demonstrate the greatest correlation with MRP mRNA expression.

Materials and Methods

Liver samples

Ninety human liver lysates, donor ages 17 years to 79, were purchased from Xenotech LLC (Lenexa, KS) in TRIzol® Reagent. Demographics of the donors included in this study are listed in Supplementary Table 1. Liver specimens were isolated from 52 males and 38 females. Of the 90 specimens, 69 were Caucasian, 9 were Hispanic, 9 were African American and 3 were of Asian descent. This study received institutional review board exemption status by the University of Kansas Medical Center Human Subjects Committee because the specimens were obtained commercially and not identified to the authors of the study.

RNA extraction

Total RNA was isolated from each sample using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. RNA was quantified by UV spectrophotometry at 260/280nm and diluted to 1µg/µl in diethyl pyrocarbamate-treated water. RNA integrity was assessed by formaldehyde-agarose gel electrophoresis, and integrity was confirmed by visualization of 18S and 28S rRNA bands.

QuantiGene® multiplex suspension array

Human liver mRNA expression was determined by the Quantigene multiplex suspension assay (Panomics Inc., Fremont, CA). Individual bead-based oligonucleotide probe sets specific for each human gene examined were developed by Panomics Inc. using previously published NCBI gene accession numbers (Supplementary Table 2). GAPDH mRNA expression was used as an internal control for each sample. Samples were analyzed by Panomics, Inc using a Bio-Plex System Array reader with Luminex 100 xMAP technology, and data was acquired using Bio-Plex Data Manager Software (Bio-Rad, Hercules, CA). Assays were performed according to the manufacturer's protocol (Panomics, Inc.). Briefly, 3 µg of total RNA was incubated overnight at 53°C with X-MAP beads containing oligonucleotide capture probes, label extenders, and blockers. The next day, beads and bound target RNA were washed and subsequently incubated with amplifier at 46°C for 1 hr. Next, samples were washed and incubated with label (biotin) and incubated at 46°C for 1 hr. Samples were washed and incubated with streptavidin-conjugated R-phycoerythrin, which binds biotinylated probes, and incubated at room temperature for 30 min. Streptavidin-conjugated R-phycoerythrin fluorescence was then detected for each analyte within each sample. All data were standardized to the internal control GAPDH. Data is expressed as the ratio of relative light units of target gene mRNA normalized to GAPDH mRNA.

Statistical analysis

GraphPad Prism (v4) (GraphPad Software, Inc, San Diego, CA) and JMP (v.7.0) (SAS Institute, Inc., Cary, NC) were used for creating graphs and conducting exploratory data analysis, respectively. Statistical tests were performed using SAS (v.9.1) (SAS Institute, Inc., Cary, NC). The following procedural statements were used in SAS: PROC CORR (linear correlations and Pearson partial correlations), PROC REG (stepwise selection of coefficients of partial determination), PROC MEANS (mean, standard error, and coefficient of variation), PROC GLM (analysis of variance with Bonferroni *post-hoc* multiple comparisons), and PROC TTEST (two level comparisons, such as gender). Linear correlations were used to determine the strength of association between MRP2-4 mRNA and

each transcription factor. This analysis was followed by stepwise regression to estimate partial R^2 values for each transcription factor in order to determine which transcription factors, among all factors tested, best explained the variability of each MRP gene. Interrelationships between the top two transcription factor genes identified by the stepwise regression were analyzed by first-order partial correlation coefficient analysis, which tests the strength of the association between one transcription factor and MRP, while controlling for variability in the second factor. Linear regressions and first-order partial correlations were deemed significant at $p < 0.005$. All other comparisons were considered statistically significant at $p < 0.05$.

Results

Distribution of MRP2-4 mRNA expression in adult human livers

The frequency distributions for MRP2-4 mRNA expression in adult human liver are unimodal (Figure 1). There were no significant differences observed in MRP expression with respect to donor gender or ethnicity (data not shown). MRP2 and 4 mRNA expression were not different by age class (17-39, 40-59, or 60+ years old); however, MRP3 mRNA expression was 1.5-fold higher in the 60+ year old cohort compared to the 17-39 year old group (data not shown).

Correlation coefficient (linear regression)

Regression analysis was performed to test the strength of linear relationships between each MRP gene and transcription factor combinations. Zero-order linear regressions are routinely used as an initial, and sometimes final, step towards elucidating potential correlations between two variables. Correlation coefficients (R-values) are shown in Figure 2. Notably, a substantial number of statistically significant correlations ($p < 0.005$) in mRNA levels between each MRP gene and the 12 transcription factors were observed. MRP2 mRNA correlated with all 12 transcription factors. The most notable correlations for MRP2 were CAR, PXR, HNF4 α , PPAR α , and LXR α . MRP3 mRNA correlated with 10 transcription factors. HNF1 α , PPAR α , PXR, HNF4 α , LXR α , and CAR exhibited the highest correlation with MRP3 mRNA. MRP4 mRNA correlated with 9 transcription factors including PPAR α , LXR α , GR, CAR, and HNF1 α .

The highest R-value for correlation with MRP2 was CAR (0.853), with MRP3 was HNF1 α (0.740), and with MRP4 was PPAR α (0.725). R-values for the remaining transcription factors were variable. Despite the conservative limit for determining statistical significance among the regressions, the linear regression procedure did not identify a single transcription factor that best correlated with MRP mRNA expression.

Coefficient of partial determination (stepwise selection of partial R^2)

In order to rank candidate regulatory genes for each transporter, stepwise regression was used to compute partial R^2 values. The stepwise procedure is a robust method of approximating a single linear function that includes multiple, statistically significant, independent variables. When the stepwise procedure is complete, the overall model R^2 value is presented as the sum of the individual partial R^2 values which are representative of each of the transcription factors included in the statistical model. The MRP2 stepwise regression model yielded the highest R^2 of 0.87, meaning that 87% of the variability in MRP2 was explained by transcription factor mRNA levels. The MRP3 and MRP4 stepwise regression models exhibited overall model R^2 values of 0.66 and 0.68, respectively.

The stepwise ranking procedure identifies the single independent variable, in this case the transcription factor, whose mRNA expression is most predictive of MRP expression relative

to all other transcription factors within the multivariate linear model. With respect to each individual MRP gene, there was one transcription factor that comparatively best explained MRP expression. To distinguish between the top two transcription factors identified in each MRP stepwise regression, the pair of transcription factors were plotted in comparison to the overall model R^2 (Figure 3). CAR was selected as the principal transcription factor (partial $R^2 = 0.73$) in the MRP2 multivariate model, whereas HNF1 α (partial $R^2 = 0.55$) and PPAR α (partial $R^2 = 0.53$) were identified as the factors that most accounted for MRP3 and MRP4 expression, respectively. PXR, RXR α , and GR were selected second in the stepwise models for MRP2, 3, and 4, respectively. Linear regressions for MRP2-4 and the most correlative transcription factors (CAR, HNF1 α and PPAR α , respectively) in 90 adult human livers are shown in Figure 3.

Upon further examination, there was no relationship between the MRP residuals and correlative transcription factor mRNA expression (data not shown). The residuals are the difference between actual MRP mRNA expression and predicted expression from the linear regression. The residual plots confirmed that the variability of MRP mRNA expression is random about the linear regression model and the residuals are not dependent on correlative transcription factor mRNA expression.

Partial correlation coefficient

First order partial correlation coefficient analysis has been used in previous studies to identify master gene regulators of various cytochrome P450 enzymes and transporters in human liver (Wortham et al. 2007). For the purpose of this study, partial correlation coefficients were calculated for the two most correlative transcription factors for each MRP gene, as identified in the stepwise selection analysis (Figure 3). Partial correlation coefficients were determined for each receptor-MRP association by statistically controlling for the variability of the second receptor. Thus, the presence of a cooperative relationship and/or co-regulation between the transcription factors was tested. For example, by holding PXR constant, the influence of PXR on the relationship between CAR and MRP2 could be determined. It is important to further explore the inter-relationships between the transcription factors as MRP expression may be influenced indirectly via receptor-receptor interactions.

Correlation coefficients (linear regressions) and partial correlation coefficients for the two transcription factors with the highest correlation to each MRP gene are presented in Figure 4. CAR and PXR correlations to MRP2 are both reduced in the respective partial correlation analyses; however, the associations did not lose statistical significance ($p < 0.005$). The same trend was observed for MRP4, where each transcription factor-transporter correlation was reduced upon holding the variability of one receptor constant, however statistical significance was unchanged. The significant correlation between MRP3 and RXR α was lost upon controlling for the variability in HNF1 α mRNA expression (R -value = 0.116, $p = 0.280$). This finding suggests that RXR α does not directly account for MRP3 variability, and that the association between RXR α and MRP3 revealed during the stepwise selection is more likely a result of the high correlation between HNF1 α and RXR α (R -value = 0.618, $p < 0.0001$).

Discussion

This manuscript investigated for the first time the influence of variation in mRNA expression of transcription factors with basal levels of hepatic MRP2-4 in 90 adult human liver specimens. Linear regression identified significant correlations between the mRNA expression of 9 to 12 transcription factors for each MRP gene. Using a stepwise selection of partial R^2 values, we were able to identify two transcription factors whose expression best

correlated with the mRNA levels of each MRP. As exhibited in Figure 3, the combined expression of CAR and PXR predicted 81% of the variability in MRP2 levels in human livers. Similarly, HNF1 α and RXR α expression accounted for 58% of the variability in MRP3 mRNA. Lastly, 59% of the variance in MRP4 expression was explained by PPAR α and GR levels. In each of these cases, one transcription factor accounted for a significantly higher percentage of the variability in the corresponding MRP mRNA expression. Subsequent analysis was undertaken to identify potential relationships between each pair of transcription factors and the cooperative ability of these two transcription factors to influence MRP levels. CAR and PXR independently correlated with MRP2. Similarly, PPAR α and GR independently correlated with MRP4. Interestingly, the correlation of the heterodimer partner RXR α and MRP3 was dependent on HNF1 α expression. This interrelationship has not been previously reported and may reflect coordinated regulation of all three genes by an unknown upstream transcription factor.

In comparison to linear regression determination, the partial R² analysis provided a clear distinction between the strength of relationships for CAR-MRP2 and PXR-MRP2. The transcription factors are directly compared to each other by combining multiple genes within a single MRP stepwise regression model. Zero-order linear regression yielded comparable correlation coefficients (R-values) for CAR-MRP2 (0.853) and PXR-MRP2 (0.826) (Figure 2), whereas the MRP2 partial R² values for CAR (0.73) and PXR (0.08) were quite different (Figure 3). The inclusion of multiple, independent variables is not permitted in the zero-order linear regression procedure. By relying solely upon linear regression analysis, we would not have been able to differentiate the strength of relationships between CAR/PXR and MRP2. Although the highest correlation coefficients corresponded with the highest partial R² value, the partial R² analysis proved to be a much more robust procedure when it came to identifying and ranking the transcription factors that best explained the variability of MRP mRNA expression. Our use of first-order partial correlations is supported, in part, in a report by de la Fuente *et al* in which the false discovery rate (independent of statistical threshold, i.e., $p = 0.01, 0.001$ or 0.0001) using zero-order Pearson correlation analysis (linear regression) was approximately 40-50%. In their study, the false discovery rate was reduced to less than 15% when first-order partial correlations were used (de la Fuente *et al.* 2004). Because there are many regulatory factors to be considered in human drug metabolism (transcription factors, enzymes, transporters), a multivariate approach using first-order partial correlations is a useful tool to differentiate and rank gene-gene relationships.

Biological data support the associations observed between mRNA levels of MRP and nuclear receptor genes. For example, the rat *Mrp2* gene contains a responsive element that is activated by co-transfection with the rat *CAR* gene (Kast *et al.* 2002). Treatment of mice with a daily dose of the CAR ligand TCPOBOP for 4 days induces hepatic mRNA expression for the CAR target, *Cyp2b10*, as well as *Mrp2* (Maher *et al.* 2005). TCPOBOP induction of *Mrp2* is absent in CAR-null mice (Huang *et al.* 2003). Little is known about the relationship between *Mrp3* and HNF1 α in rodent liver. However, HNF1 α -null mice exhibit no basal differences in hepatic *Mrp3* expression, suggesting that HNF1 α may act through indirect means to regulate MRP3, or alternatively that constitutive rodent and human MRP3 are regulated via distinct pathways (Maher *et al.* 2006). As our data in Figure 4 suggest, the high correlation of MRP3 and HNF1 α likely results from the strong interaction between HNF1 α and RXR α . Lastly, there is evidence in mice that PPAR α can regulate expression of *Mrp4*. The mouse *Mrp4* gene contains multiple DR-1 elements in its upstream promoter region (Moffit *et al.* 2006). Treatment of mice with peroxisome proliferators, such as clofibrate and perfluorodecanoic acid, induces expression of *Mrp4* mRNA and protein (Maher *et al.* 2005; Moffit *et al.* 2006). Using knockout mice, up-regulation of *Mrp4* mRNA after clofibrate was shown to be dependent upon PPAR α expression. Our analysis is

particularly important because it not only confirms, but also extends prior research in rodent and *in vitro* models by comprehensively reviewing gene expression pathways in a relatively large database of human livers. The transcription factors identified as highly correlative to each MRP gene using stepwise selection may be biologically relevant.

There are some limitations of extrapolating rodent to human transcription factors and should be taken into consideration. Although the rodent and human findings are similar, it should be noted that the majority of the rodent data is generated using xenobiotic induction. Little is known about the dietary, environmental, and/or pharmaceutical exposure of humans from which the liver specimens were obtained. In addition, transcription factor function between human and rodent isoforms can be different. For example, transcriptional activation by rodent and human PXR show preferential responses to different sets of xenobiotics (rodent: pregnane 16 α -carbonitrile, human: rifampicin) (Jones et al. 2000). There are also clear differences in rodent and human PPAR α signaling. Whereas PPAR α activation in mice causes peroxisome proliferation, increased liver weight, and liver carcinogenesis, human PPAR α activation is not associated with these sequelae (Gonzalez and Shah 2008).

It is worth noting that the stepwise regression models did not select Nrf2 or AhR as a top transcription factor for any MRP gene. Although Nrf2 and AhR were included in the final stepwise models for MRP2 and MRP4, respectively, the partial R² values were too low for further analysis using partial correlation coefficients. Possible AhR and Nrf2 co-regulation of transporter genes, such as Mrps, is an interesting research topic that has recently been reviewed (Kohle and Bock 2007). However, reports of Nrf2 or AhR mediated gene induction are commonly based on studies where rodents or cells are administered prototypical inducers such as oltipraz or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, respectively.

Recent work from our laboratory and others has identified a critical role for Nrf2 in regulating the inducible expression of mouse Mrp2-4 genes. This conclusion was based upon *in vitro* chromatin immunoprecipitation of Nrf2 bound to antioxidant response elements in the upstream promoter regions of these genes, along with *in vivo* experiments demonstrating the up-regulation of Mrp2-4 mRNA and protein in livers of wild-type mice treated with the Nrf2 activator, oltipraz. Importantly, inducible expression was lost in Nrf2-null mice (Maher et al. 2007). Likewise, increased levels of Mrp3 and Mrp4 mRNA and protein were observed in wild-type mice treated with a hepatotoxic dose of acetaminophen, with no change in Nrf2-null mice (Aleksunes et al. 2008). Given the data generated in this manuscript along with rodent studies it can be hypothesized that Nrf2 is more important in regulating the inducible expression of some MRP/Mrp genes rather than constitutive levels as studied in this human liver study. An alternative explanation for this discordance may be differences in regulation between species.

Co-regulation of nuclear receptor and MRP mRNA levels by upstream transcription factors is an alternate interpretation of these data. Recent work suggests that drug metabolic and excretion pathways are coordinately regulated. Therefore, it is possible that the relationships identified between MRP2-CAR, MRP3-HNF1 α , and MRP4-PPAR α are an extension of this coordination. Additional experimentation is necessary to dissect the direct and indirect signaling pathways in human hepatocytes and identify potential upstream regulatory mechanisms for the nuclear receptor-MRP pairs.

There are a number of advantages with the methods employed in the current study. Use of the stepwise selection of partial R² values is a novel method that was able to discriminate amongst numerous transcription factors that appeared to predict MRP mRNA levels based upon linear regression. We conducted a second multivariate analysis including two likely unrelated genes (steroid receptor coactivator-1, delta-aminolevulinic acid synthase 1) in addition

to the various transcription factors. Inclusion of these variables in the multivariate analysis did not alter the stepwise selection of CAR, HNF1 α , and PPAR α , for MRP2, 3, and 4, respectively (unpublished observations). This suggests that the approach presented in this manuscript can discriminate variables when unlikely correlates are included. In addition, this unbiased study included all 90 samples with diversity in gender, age, and ethnicity. Although basic demographic information was provided for the donors, undiagnosed comorbid conditions as well as xenobiotic induction are potential confounders to the data interpretation that cannot be controlled.

Spliced transcripts and allelic variants have been previously identified for CAR, PPAR α , and HNF1 α (Ringeisen et al. 1993; Auerbach et al. 2003; Flavell et al. 2005). The presence of these variants in the current panel of 90 liver samples was not investigated and could explain some variability in expression of MRP2-4. Polymorphisms in a particular *MRP* gene may have also impacted our analysis (Lee et al. 2004; Meier et al. 2006; Fukushima-Uesaka et al. 2007). For example, two polymorphisms in *MRP2* (V1188E, C1515Y) are associated with increased expression of MRP2 in human liver (Meier et al. 2006). The presence of these variants in this human liver panel is one possible explanation for specimens that did not show correlation between CAR and MRP2 mRNA levels.

It is the intent of this project to identify correlations between levels of transcription factors and MRP genes with the long-term goal to better predict an individual's response to drug therapy (both efficacy and side effects). Future studies need to determine whether the identified transcription factor:MRP relationships occur via direct gene activation because a causal relationship cannot be definitively made from the current analysis. Along these lines, the protein expression and functional activity of MRP2-4 in panels of liver specimens need to be quantified in order to correlate nuclear receptor mRNA expression with transport activity. The methods used in the current study may serve as a novel approach for additional investigations into the regulation of biotransformation enzymes as well as other hepatobiliary transporters in normal adult human liver.

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Non-Standard Abbreviations

AhR	aryl hydrocarbon receptor
CAR	constitutive androstane receptor
FXR	farnesoid X receptor
GR	glucocorticoid receptor
HNF	hepatocyte nuclear factors
LXR	liver X receptor
MRP	multidrug resistance-associated proteins
Nrf2	nuclear factor E2-related factor 2
PPAR	peroxisome proliferator activated receptor
PXR	pregnane X receptor
RXR α	retinoid X receptor alpha

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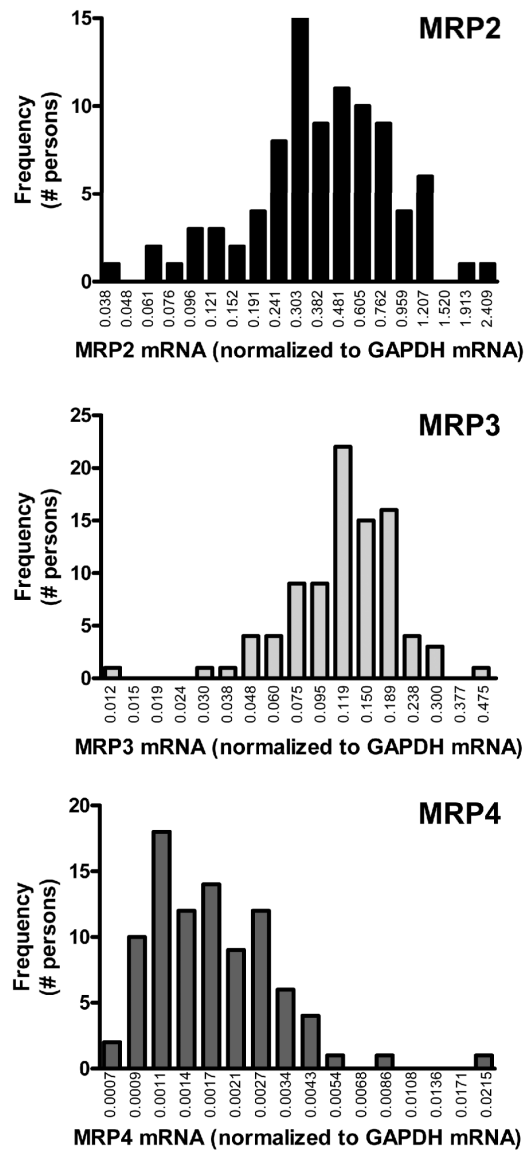


Figure 1. Frequency distribution of MRP2-4 mRNA in adult human livers mRNA levels of MRP2-4 transporters are presented as frequency distributions on a logarithmic scale normalized to GAPDH mRNA.

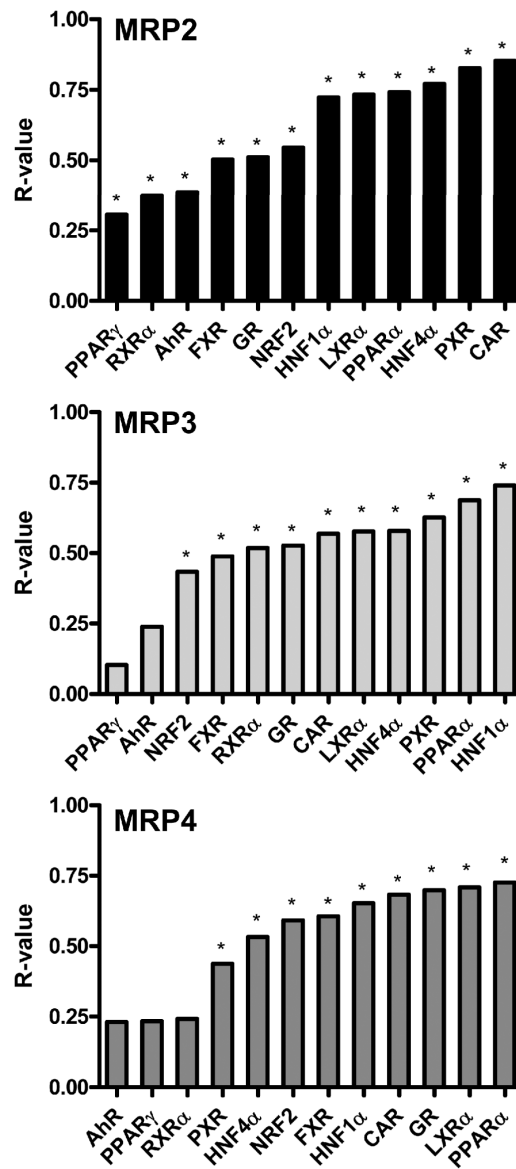


Figure 2. Correlation of MRP2-4 and transcription factor mRNA expression
 Comparisons between each MRP gene and a panel of transcription factors were determined by linear regression and presented as R-values. Statistical significance (*) was set at $p < 0.005$.

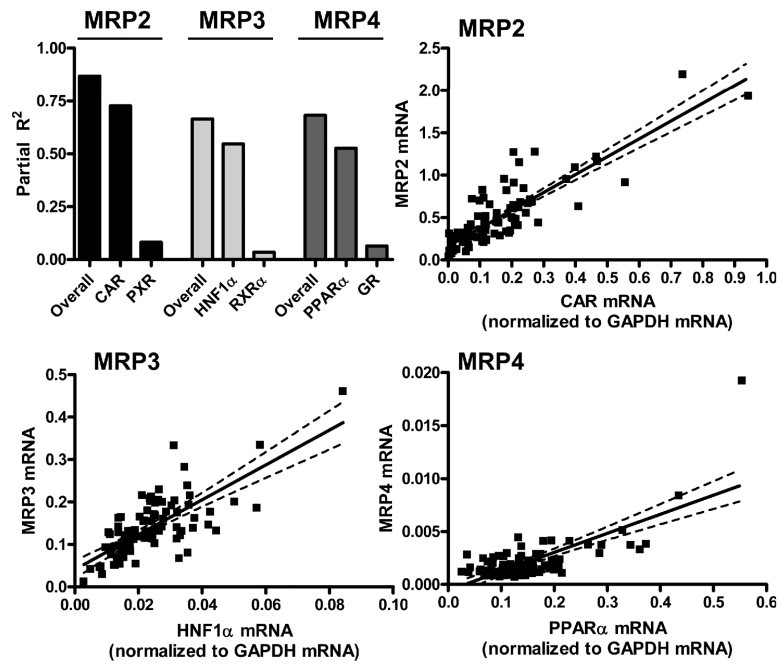


Figure 3. MRP:transcription factor interactions

Correlative transcription factors were ranked using stepwise selection and the factors with the highest partial R^2 values are presented. Partial R^2 values for each transcription factor are plotted in comparison to the overall model R^2 on a scale of 0-1.0. Linear regressions for MRP2-CAR, MRP3-HNF1 α , and MRP4-PPAR α mRNA are shown. Data are presented as mean mRNA expression (normalized to GAPDH mRNA). Confidence intervals (95%) are depicted as dashed lines.

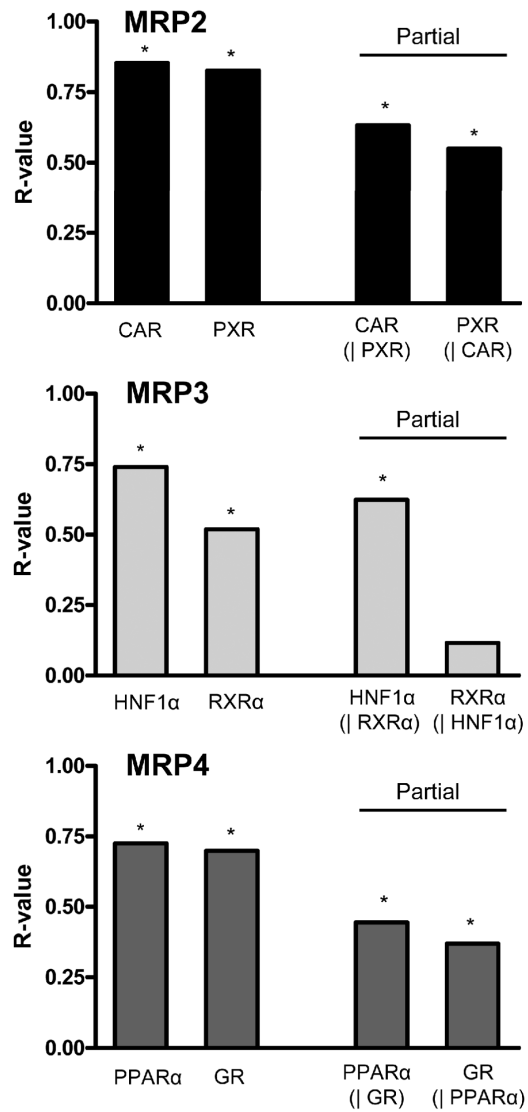


Figure 4. Transcription factor interrelationships and correlation to MRP2-4 mRNA

Correlation coefficients (R-values) obtained from linear regression analysis in Figure 1 are presented for MRP2 (CAR and PXR), MRP3 (HNF1 α and RXR α), and MRP4 (PPAR α and GR) mRNA. Partial correlation coefficients (R-values) were calculated for each MRP-transcription factor association, controlling for variability in the second transcription factor. The symbol | denotes the partial variable. Statistical significance (*) was set at $p < 0.005$.

Table 1

Sample demographics (Xenotech, Lenexa, KS)

Gender	
Male	52
Female	38

Ethnic Descent	
Caucasian	69
African American	9
Hispanic	9
Asian	3

Age category (years)	
17-39	19
40-59	46
60+	25

Table 2

Gene accession numbers

Gene	Species	Accession Number	Symbol
MRP2	Human	NM_000392	ABCC2
MRP3	Human	NM_003786	ABCC3
MRP4	Human	NM_005845	ABCC4
AhR	Human	NM_001621	ARR
CAR	Human	NM_005122	NR1I3
FXR	Human	NM_005123	NR1H4
GR	Human	NM_000176	NR3C1
HNF1 α	Human	NM_000545	TCF1
HNF4 α	Human	NM_000457	HNF4A
LXR α	Human	NM_005693	NR1H3
Nrf2	Human	NM_006164	NFE2L2
PPAR α	Human	NM_005036	PPARA
PPAR γ	Human	NM_138712	PPARG
RXR α	Human	NM_002957	RXRA
PXR	Human	NM_003889	NR1I2
GAPDH	Human	NM_002046	GAPD