Genetic Polymorphisms in the TATA Box and Upstream Phenobarbital-Responsive Enhancer Module of the UGT1A1 Promoter Have Combined Effects on UDP-Glucuronosyltransferase 1A1 Transcription Mediated by Constitutive Androstane Receptor, Pregnan X Receptor, or Glucocorticoid Receptor in Human Liver

Ye Li, David Buckley, Shuang Wang, Curtis D. Klaassen, and Xiao-bo Zhong

Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas (Y.L., D.B., C.D.K., X.Z.); and Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, New York (S.W.)

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ABSTRACT:

Transcription of UDP-glucuronosyltransferase (UGT) 1A1 is regulated by the transcription factors, constitutive androstane receptor (CAR), pregnane X receptor (PXR), glucocorticoid receptor (GR), hepatocyte nuclear factor (HNF) 1α, and HNF4α. The purpose of this study was to determine whether the genetic polymorphisms in the TATA box and upstream phenobarbital-responsive element module (PBREM) of the UGT1A1 promoter have combined effects on UGT1A1 transcription mediated by the transcription factors. A polymorphism of A(TA)5–8TAA in the PBREM was genotyped in 98 human liver samples. Relative mRNA levels of CAR, PXR, GR, HNF1α, HNF4α, and UGT1A1 were quantified by a multiplex branched DNA technique. Correlations of mRNA levels between UGT1A1 and the transcription factors were established in liver samples with different combined genetic polymorphisms. Correlation of mRNA levels between UGT1A1 and CAR, PXR, or GR, but not HNF1α or HNF4α, was abolished in the samples with the combined genotype of TA7/7 plus −3279G/G, which was also associated with significantly lower UGT1A1 mRNA levels compared with other combined genotypes. Correlations of mRNA levels between UGT1A1 and CAR or PXR were reduced but not abolished in the samples with the combined genotype of TA6/7 plus −3279 G/G, which showed significantly lower UGT1A1 mRNA levels compared with the combined genotype of TA6/6 plus −3279/−3279 G/G, and other genotypes containing TA6/6. In conclusion, the combined genotypes containing (TA)5–8TAA and −3279 G/G decrease UGT1A1 transcription mediated by CAR, PXR, or GR but not by HNF1α or HNF4α.

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ABBREVIATIONS: Pol II, polymerase II; TSS, transcriptional start site; DPE, downstream promoter element; UGT1A1, UDP-glucuronosyltransferase 1A1; PBREM, phenobarbital-responsive enhancer module; CAR, constitutive androstane receptor; PXR, pregnane X receptor; GR, glucocorticoid receptor; SNP, single nucleotide polymorphism; HNF, hepatocyte nuclear factor; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; PCR, polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LD, linkage disequilibrium.
enhancer and core promoter are important for enhancing gene transcription by transcription factors. Genetic polymorphisms in these elements may have a significant influence on gene expression. UGT1A1 is a gene that has polymorphisms in both the core promoter and enhancer elements.

UGT1A1 is a phase II drug-metabolizing enzyme, which is essential for the metabolism of bilirubin and some drugs. The core promoter in the UGT1A1 gene contains a typical Pol II core promoter structure. A TATA box (TATAA) is located at -28 from the TSS, and a DPE with an AGTCC is found at +26 from the TSS (Fig. 1A). A dinucleotide polymorphism of A(TA)$_{6,8}$TAA in the TATA box is common in all ethnic populations, with A(TA)$_{6}$TAA as the major allele (Beutler et al., 1998). Minor alleles with higher TA repeat copy numbers, A(TA)$_{7}$TAA and A(TA)$_{8}$TAA, have a lower affinity to bind the transcription factor II D-binding protein and thus result in less UGT1A1 transcription (Hsieh et al., 2007). These alleles have been associated with Gilbert’s syndrome (hyperbilirubinemia) (Aono et al., 1995; Bosma et al., 1995; Monaghan et al., 1996), as well as toxicity in cancer chemotherapy patients treated with irinotecan (Iyer et al., 2002; Innocenti et al., 2004; Marcuello et al., 2004).

An enhancer element, referred to as the phenobarbital-responsive enhancer module (PBREM), is located at ~3.2 kilobases upstream of the UGT1A1 TSS (Sugatani et al., 2001). PBREM serves as a binding site for constitutive androstane receptor (CAR) (Sugatani et al., 2004), pregnane X receptor (PXR) (Sugatani et al., 2004), and glucocorticoid receptor (GR) (Sugatani et al., 2005; Usui et al., 2006a) (Fig. 1B). Transcription of UGT1A1 can be enhanced by the binding of these transcription factors to the PBREM element. A common genetic polymorphism, -3279T>G, has been identified in PBREM, which is associated with a decrease of UGT1A1 transcription (Sugatani et al., 2002). Individuals carrying the -3279G allele have a higher risk for Gilbert’s syndrome (Sugatani et al., 2001; Sun et al., 2006) and irinotecan toxicity (Kitagawa et al., 2005).

By transfection of promoter-luciferase plasmids into HepG2 cells, Sugatani et al. (2008) showed that enhancement of transcription by CAR, PXR, or GR, or aryl hydrocarbon receptor ligands was reduced in a construct containing both A(TA)$_{6,8}$TAA and -3279G polymorphisms, compared with constructs with other combined polymorphisms. The purpose of the current study was to determine whether the TA polymorphism in the TATA box and the SNP (-3279T>G) in the PBREM element have combined effects on UGT1A1 transcription mediated by the transcription factors in human liver samples. Negative controls include three SNPs [-3156G>A, -2353C>T, and -997G>A] that are not located in the TATA box or the PBREM element and hepatocyte nuclear factor (HNF) 1$\alpha$ and HNF4$\alpha$ that enhance UGT1A1 transcription by binding to other enhancers (Usui et al., 2006b; Aueviriyavit et al., 2007), as well as a transcriptional coactivator of peroxisome proliferator-activated receptor $\alpha$ (PGC1$\alpha$) that does not bind to the UGT1A1 promoter.

Materials and Methods

Human Liver Samples. Human liver tissue ($n = 98$) was purchased from XenoTech, LLC (Lenexa, KS) for DNA and RNA isolation. The liver samples were acquired by XenoTech, LLC through the Midwest Transplant Network (Westwood, KS), the National Disease Research Interchange (Philadelphia, PA), and the Anatomical Gift Foundation (Woodbine, GA). Livers were initially harvested for liver transplantation but were not used for various reasons and were subsequently donated for research. The livers were immediately cooled after procurement with a cold perfusion solution and stored at -80°C. All liver samples were tested for and declared free of infectious agents, including human immunodeficiency virus, hepatitis B, and hepatitis C. Demographic information such as gender, age, ethnicity, and confounding factors is provided in Supplemental Table S1.

Quantification of mRNAs. mRNA levels of UGT1A1 and its transcription factors were quantified by a QuantiGene Plex 2.0 assay (Panomics, Fremont, CA) instead of a standard real-time-PCR assay, because QuantiGene Plex 2.0 has measurement accuracy comparable to that of the standard real-time-PCR technique (Canales et al., 2006) but easier for multiplexing. Total RNA from each liver tissue sample was isolated using TRIzol reagent according to the manufacturer’s protocol (Invitrogen, Carlsbad, CA). Levels of gene transcription were determined by a QuantiGene Plex 2.0 assay, which combined branched DNA signal amplification technology and xMAP (multianalyte profiling) bead technology to enable simultaneous quantification of multiple RNA targets directly from liver tissue samples. Individual, bead-based, oligonucleotide probe sets specific for each human mRNA transcript were designed and synthesized by Panomics for detecting UGT1A1 [NM_000463 (National Center for Biotechnology Information mRNA reference sequence number) and the transcription factors CAR (NM_005122), PXR (NM_003889), GR (BC015610), HNF1$\alpha$ (NM_000545), HNF4$\alpha$ (Z49825), PGC1$\alpha$ (NM_013261)] as a transcriptional coactivator not related to UGT1A1 transcription. GAPDH (NM_002046) was used as a reference for normalizing gene transcription. The probe sets of capture probe, capture extender, blocker, and label extender were designed for specific recognition of each RNA transcript without cross-hybridization. Each capture probe was immobilized to capture beads with a specific fluorescent spectrum, which are...
TABLE I

Distribution and correlation of gene transcription levels between UGT1A1 and the transcription factors, CAR, PXR, GR, HNF1α, and HNF4α

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Highest % normalized with GAPDH</th>
<th>Lowest</th>
<th>Mean</th>
<th>S.D.</th>
<th>Mean after Log Transformation*</th>
<th>S.D. after Log Transformation</th>
<th>Pearson’s Correlationb</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>84.7</td>
<td>1.7</td>
<td>20.8</td>
<td>17.9</td>
<td>1.180</td>
<td>0.352</td>
<td>0.695 &lt;0.001</td>
</tr>
<tr>
<td>CAR</td>
<td>94.4</td>
<td>0.3</td>
<td>16.7</td>
<td>17.4</td>
<td>0.975</td>
<td>0.546</td>
<td>0.602 &lt;0.001</td>
</tr>
<tr>
<td>PXR</td>
<td>51.5</td>
<td>0.3</td>
<td>12.6</td>
<td>10.4</td>
<td>0.930</td>
<td>0.437</td>
<td>0.398 &lt;0.001</td>
</tr>
<tr>
<td>GR</td>
<td>40.7</td>
<td>3.7</td>
<td>10.4</td>
<td>6.0</td>
<td>0.967</td>
<td>0.191</td>
<td>0.558 &lt;0.001</td>
</tr>
<tr>
<td>HNF1α</td>
<td>8.4</td>
<td>0.3</td>
<td>2.4</td>
<td>1.3</td>
<td>0.832</td>
<td>0.234</td>
<td>0.565 &lt;0.001</td>
</tr>
<tr>
<td>HNF4α</td>
<td>134</td>
<td>6.9</td>
<td>35.2</td>
<td>19.8</td>
<td>1.493</td>
<td>0.216</td>
<td>0.343</td>
</tr>
<tr>
<td>PGC1α</td>
<td>58.0</td>
<td>0.7</td>
<td>7.8</td>
<td>8.6</td>
<td>1.457</td>
<td>0.304</td>
<td>0.095</td>
</tr>
</tbody>
</table>

* Log transformation: X = log100X; X, relative mRNA level after normalization with GAPDH.

Pearson’s correlation of mRNA levels between UGT1A1 and the nuclear receptors commercially available from Panomics. Assays were performed according to the Panomics protocol. In brief, 3.0 μg of human liver total RNA was incubated overnight at 53°C with gene-specific nMAP beads containing oligonucleotide capture probes, capture extenders, label extenders, and blockers. The beads and bound target RNAs were washed with wash buffer and subsequently incubated with amplifier reagents at 46°C for 1 h. The samples were then incubated with biotinylated labeling probes at 46°C for an additional 1 h. The beads were washed with wash buffer and incubated with streptavidin-conjugated R-phycocerythrin at room temperature for 30 min. Streptavidin-conjugated R-phycocerythrin fluorescence was then detected for each mRNA transcript using a Luminex instrument (Luminex, Austin, TX). Data acquisition was performed using Bio-Plex Data Manager Software (Bio-Rad Laboratories, Hercules, CA). All data were normalized to the internal control GAPDH and expressed as a ratio of GAPDH mRNA.

Genotyping of the UGT1A1 Polymorphisms. Genomic DNA was isolated from liver tissue samples using the ChargeSwitch gDNA Mini Tissue Kit (Invitrogen), following the manufacturer’s protocol. Targets for genotyping the UGT1A1 polymorphisms were amplified by PCR. PCR primers were designed using DS Gene Software (Accelrys, Cambridge, UK) and the sequences are listed in Supplemental Table S2. Primers were synthesized by Integrated DNA Technologies (Coralville, IA), and the subsequent PCR reactions were performed using GoTag DNA Polymerase (Promega, Madison, WI), with cycling conditions of 95°C for 3 min, 40 cycles of 94°C for 15 s, 60°C for 30 s, and 68°C for 45 s, followed by 68°C for 5 min.

Genotypes of the A(TA)5–8TAA polymorphism were determined by bidirectional sequencing with forward or reverse primers. PCR products were purified with a Prescreening Kit (USB, Cleveland, OH). DNA sequencing was carried out with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). For dye terminator removal, PERFORMA DTR Gel Filtration cartridges (Edge Biosystems, Gaithersburg, MD) were used. Sequences were analyzed with a 3130 DNA Analyzer (Applied Biosystems).

The promoter SNPs were genotyped on thin-film biosensor chips using technology described previously (Nakamoto et al., 2007). Sequences for capture probes (P1) and detection probes (P2) are listed in Supplemental Table S3. Linkage disequilibrium (LD) among the genetic polymorphisms was analyzed by HaploView (version 4.0). An LD block is created by confidence intervals if 95% of the informative comparisons are in strong LD, using default algorithms of 95% confidence bounds on D’.

Statistical Analysis. Multiple linear regressions were used to assess the effects of confounding factors on UGT1A1 gene transcription, including age, sex, ethnicity, reasons of death, and smoking and drinking history. Influence of the UGT1A1 genetic polymorphisms on UGT1A1 gene transcription was examined by a two-sample t test with pairwise comparison of the mean values of UGT1A1 mRNA levels between different UGT1A1 genotype subgroups. The correlations between UGT1A1 and the transcription factors were determined by Pearson’s correlation coefficient (JMP version 7.0; SAS Institute, Cary, NC).

Results

Association of Gene Transcription between UGT1A1 and the Transcription Factors, CAR, PXR, GR, HNF1α, and HNF4α. Table 1 summarizes the highest, lowest, mean, and S.D. of mRNA levels of UGT1A1 and the transcription factors, CAR, PXR, GR, HNF1α, and HNF4α, in human livers. Marked interindividual variations in the relative mRNA levels of UGT1A1 and the transcription factors were found in the liver samples. After the log transformation of mRNA levels, the distribution is more normally distributed. For example, Fig. 2 shows a normal distribution of UGT1A1 mRNA levels in the study population.

A Pearson’s correlation analysis was used to establish associations of gene transcription between UGT1A1 and the transcription factors, CAR, PXR, GR, HNF1α, and HNF4α. PGC1α, which does not directly interact with the UGT1A1 promoter, served as a negative control. Pearson’s correlation indicates that the mRNA levels of all transcription factors examined, except PGC1α, were associated with the mRNA level of UGT1A1 (p < 0.001).

Influence of Genetic Polymorphisms on UGT1A1 Transcription. Genotypes of the A(TA)5–8TAA polymorphism and the SNPs in the UGT1A1 promoter were obtained by two different genotyping methods. Figure 3A shows genotyping results of the A(TA)5–8TAA polymorphism by sequencing. Five different genotypes of the A(TA)5–8TAA polymorphism, TA6/6, TA6/7, TA7/7, TA5/6, and TA7/8, were observed in the study population with frequencies shown in the lower panel of Fig. 3A. TA6/6 and TA7/6 are the two common genotypes of the A(TA)5–8TAA polymorphism, accounting for 47 and 44%, respectively. The frequency of homozygous TA7/7 is 6% in the study population, which is slightly lower than the frequency in Caucasian
casians reported in other studies (Innocenti et al., 2002; Liu et al., 2007). TA5/6 and TA7/8 were rare, 2 and 1%, respectively. Therefore, in the following data analyses, the two samples with TA5/6 were included in the group of TA6/6, and the sample with TA7/8 was added to the TA7/7 group.

Figure 3B shows the genotyping results of the four SNPs in the UGT1A1 promoter on thin-film biosensor chips. Chips were printed in a format as noted in the most left of the first row (chip format). On each chip, eight dots in the top row were a biotinylated probe (5'-aldehyde-AAAAAAAAAAAAAAAAAAAAAAAAAA-biotin-3'), serving as a positive quality control for the anti-biotin antibody and its color-generating substrate. For each SNP, capture probes of the major alleles were spotted four times on the left side and minor alleles on the right side. In a control experiment, a negative control (no targets) and two positive controls (synthetic targets of all major alleles or all minor alleles) were applied, and specificity for each genotype was obtained.

![Genotyping results of selected individual liver samples](image)

**TABLE 2**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Regression Coefficient Estimate</th>
<th>S.E.</th>
<th>t Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.33</td>
<td>0.67</td>
<td>-0.49</td>
<td>0.62</td>
</tr>
<tr>
<td>Gender: male vs. female</td>
<td>-0.14</td>
<td>0.22</td>
<td>-0.65</td>
<td>0.51</td>
</tr>
<tr>
<td>Age</td>
<td>0.01</td>
<td>0.06</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Ethnicity: AA vs. A</td>
<td>-0.14</td>
<td>0.67</td>
<td>-0.22</td>
<td>0.83</td>
</tr>
<tr>
<td>Ethnicity: C vs. A</td>
<td>-0.02</td>
<td>0.59</td>
<td>-0.04</td>
<td>0.96</td>
</tr>
<tr>
<td>Ethnicity: H vs. A</td>
<td>0.13</td>
<td>0.65</td>
<td>0.21</td>
<td>0.84</td>
</tr>
<tr>
<td>Death: AA vs. A</td>
<td>1.14</td>
<td>1.04</td>
<td>1.10</td>
<td>0.27</td>
</tr>
<tr>
<td>Death: CVA vs. A</td>
<td>0.54</td>
<td>0.33</td>
<td>1.64</td>
<td>0.10</td>
</tr>
<tr>
<td>Death: HT vs. A</td>
<td>0.39</td>
<td>0.37</td>
<td>1.05</td>
<td>0.29</td>
</tr>
<tr>
<td>Death: MI vs. A</td>
<td>0.14</td>
<td>0.65</td>
<td>-0.23</td>
<td>0.82</td>
</tr>
<tr>
<td>Death: MVA vs. A</td>
<td>-0.04</td>
<td>0.74</td>
<td>-0.06</td>
<td>0.95</td>
</tr>
<tr>
<td>Smoking: yes vs. no</td>
<td>0.09</td>
<td>0.22</td>
<td>0.40</td>
<td>0.69</td>
</tr>
<tr>
<td>Alcohol: yes vs. no</td>
<td>0.16</td>
<td>0.22</td>
<td>0.70</td>
<td>0.48</td>
</tr>
<tr>
<td>Genotypes: TA6/7 vs. TA6/6</td>
<td>0.03</td>
<td>0.23</td>
<td>-0.14</td>
<td>0.89</td>
</tr>
<tr>
<td>TA7/7 vs. TA6/6</td>
<td>-1.30</td>
<td>0.39</td>
<td>3.30</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Ethnicity: A, Asian; AA, African-American; C, Caucasian; H, Hispanic; death: A, anoxia; AA, aortic aneurysm; CVA, cerebrovascular aneurysm; HT, head trauma; MI, myocardial infarction; MVA, motor vehicle accident.

Fig. 3. Genotypes of the UGT1A1 A(TA)₅₋₈TAA dinucleotide polymorphism by direct sequencing (A) and the four SNPs on thin-film biosensor chips (B) in the study population. Obs., observed; Pred., predicted; freq., frequency; HW, Hardy-Weinberg.
Genotypes of the four SNPs in each liver sample were recorded, based on appearance of positive signals (dark color). Four representative chip images are shown in the second row of Fig. 3B. The frequency of each SNP genotype is summarized in Fig. 3B. Similar results have been reported in other studies (Innocenti et al., 2002; Liu et al., 2007). All of the SNPs follow the Hardy-Weinberg distribution rule ($p > 0.05$). Alleles of $-3279G$ and $-3156A$ are common in the study population with a frequency of 46 and 32%, respectively. A linkage disequilibrium analysis was performed among the polymorphisms using the HaploView program with the confidence interval method, but no linkage disequilibrium block could be defined in the population, and thus no haplotype could be assigned to each subject.

A multiple linear regression analysis was applied to find factors contributing to the interindividual variation of UGT1A1 gene transcription. The factors analyzed included gender, age, ethnicity, reasons of death, smoking and drinking history, and genetic polymorphisms. As an example, Table 2 summarizes the regression coefficient, S.E., test statistic value ($t$ value), and $p$ values. Except for the genetic polymorphism of A(TA)$_5$TAA, none of the possible confounding factors examined significantly influence UGT1A1 mRNA levels ($p > 0.05$). Samples with TA$_7$/7 (including TA$_7$/8) had significantly lower mRNA levels of UGT1A1 than samples with TA$_6$/6 (including TA$_5$/6) at $p < 0.01$ after adjusting for all potential factors, whereas samples with TA$_6$/7 are not significantly different from samples with TA$_6$/6 on mRNA levels of UGT1A1 after adjusting for all potential factors ($p = 0.89$).

Because no potential confounders are significantly associated with
the UGT1A1 mRNA level, the influence of the genetic polymorphisms on UGT1A1 mRNA levels was examined by a two-sample t test with pairwise comparison of the mean values of UGT1A1 mRNA levels between different subgroups of genotypes (Fig. 4) or combined genotypes (Fig. 5). Individuals carrying TA7/7 have significantly lower UGT1A1 mRNA levels than individuals carrying TA6/6 and TA6/7 ($p < 0.01$), but the difference between individuals with the TA6/7 and TA6/6 genotypes was not statistically significant ($p = 0.93$). Individuals carrying $-3279G/G$ have significantly lower UGT1A1 mRNA levels than individuals carrying $-3279 T/G$ ($p < 0.05$), but the difference between individuals with the $-3279G/G$ and $-3279T/T$ genotypes was not statistically significant ($p = 0.09$). Individuals carrying $-3156G/A$ have significantly lower UGT1A1 mRNA levels than individuals carrying $-3156G/G$ or $-3156G/A$ ($p < 0.05$). SNPs of $-2353C/T$ and $-997G/A$ do not influence the UGT1A1 mRNA levels ($p > 0.05$).

Because no linkage disequilibrium blocks and haplotypes could be defined among the polymorphisms in the population, the influence of the genetic polymorphisms on UGT1A1 mRNA levels was further examined in subgroups with combined genotypes of $\text{A(TA)}_n\text{TA}A$ and $-3279T>G$ or between $\text{A(TA)}_n\text{TA}A$ and $-3156G>A$. Figure 5 shows the box plot of UGT1A1 mRNA levels with means and S.D.s in subgroups with different combined genotypes of $\text{A(TA)}_n\text{TA}A$ and $-3279T>G$ or between $\text{A(TA)}_n\text{TA}A$ and $-3156G>A$. Six different combined genotypes of $\text{A(TA)}_n\text{TA}A$ and $-3279T>G$ were found (Fig. 5A). The samples with TA7/7 plus $-3279G/G$ have significantly lower UGT1A1 mRNA levels than the samples with TA6/6 plus $-3279T/T$, TA6/6 plus $-3279T/G$, TA6/6 plus $-3279G/G$, and TA6/7 plus $-3279T/G$ ($p < 0.01$). However, no significant difference in UGT1A1 mRNA levels is shown between TA6/7 plus $-3279G/G$ and TA 7/7 plus $-3279 G/G$ ($p = 0.08$). In contrast, the samples with TA6/7 plus $-3279G/G$ have significantly lower UGT1A1 mRNA levels than the samples with TA6/6 plus $-3279T/T$, TA6/6 plus $-3279T/G$, TA6/6 plus $-3279G/G$, and TA6/7 plus $-3279T/G$ ($p < 0.05$).

Five different combinational genotypes of $\text{A(TA)}_n\text{TA}A$ and $-3156G>A$ were found in the study population (Fig. 5B). Only samples with TA7/7 plus $-3156G/A$ have significantly lower UGT1A1 mRNA levels than the other four combinational genotypes ($p < 0.01$). It is noteworthy that although no linkage disequilibrium block can be defined among these polymorphisms, all individuals in the study population who have TA7/7 also simultaneously carry $-3279G/G$ and $-3156A/A$.

### Effects of Genetic Polymorphisms on the Correlation of Gene Transcription between UGT1A1 and Transcription Factors

Although significant correlations of mRNA levels exist between the transcription factors examined and UGT1A1 in the study population, the levels of significance are different in samples with different genotypes of $\text{A(TA)}_n\text{TA}A$, $-3279T>G$, or $-3156G>A$ (Table 3). Three different cases were found. In case I, the association of gene transcription between UGT1A1 and CAR, PXR, GR, or HNF1 decreased in genotypes groups, in an order of homozygous major alleles > heterozygous > homozygous minor alleles. Association was not significant ($p > 0.05$) between UGT1A1 and CAR or PXR in the samples that carry TA7/7 or $-3156A/A$ or between UGT1A1 and GR in the samples of TA7/7, TA6/7, $-3279G/G$, or $-3156A/A$. In case II, mRNA levels were significantly associated between UGT1A1 and HNF4a in all $\text{UGT1A1}$ genotype groups. In case III, mRNA levels were not associated between UGT1A1 and PGC1α for all genotype groups.

### Correlations of mRNA levels between UGT1A1 and the transcription factors, CAR, PXR, GR, HNF1a, or HNF4a, were further examined in subgroups with various combined genotypes of $\text{A(TA)}_n\text{TA}A$ and $-3279T>G$ or $-3156G>A$ (Fig. 6). Three cases were found: 1) association of gene transcription between UGT1A1 and CAR (Fig. 6A) or PXR (Fig. 6B) was abolished in the samples with TA7/7 plus $-3279G/G$ ($p > 0.05$), reduced but not abolished in the samples with TA6/7 plus $-3279G/G$ ($p < 0.05$), and remained significant in the samples with TA6/7 plus $-3279T/G$ and any samples containing TA6/6 ($p < 0.05$); 2) association of gene transcription between UGT1A1 and GR (Fig. 6C) was abolished in the samples with TA7/7 plus $-3279G/G$, TA6/7 plus $-3279G/G$, and TA6/7 plus $-3279T/G$ ($p > 0.05$), but remained significant in samples containing TA6/6 ($p < 0.01$); and 3) association of gene transcription between UGT1A1 and HNF4a (Fig. 6D, similar between UGT1A1 and HNF1α; data not shown) remained significant in all samples, at various levels of significance.

### Discussion

In the current study we examined the effects of genetic polymorphisms in the UGT1A1 promoter on UGT1A1 transcription mediated by the transcription factors, CAR, PXR, GR, HNF1α, or HNF4α, in
In the study population with different combined genotypes of A(TA)_{5–8}TAA and −3279T>G, liver samples with −3279T/G (p < 0.05) but have no significant difference from liver samples with −3279T/T (p = 0.09). SNPs of −2353C>T and −997G>A have no effects on UGT1A1 mRNA levels in this liver sample population. These findings are consistent with previous reports on the effects of these genetic polymorphisms on UGT1A1 transcription (Ramírez et al., 2008), UGT1A1 activity and protein expression in liver microsomes (Peterkin et al., 2007), bilirubin metabolism (Sugatani et al., 2002), and drug metabolism of irinotecan (Innocenti et al., 2002; Kitagawa et al., 2005).

Although the −3156G>A polymorphism has a significant effect on UGT1A1 mRNA levels, further examination on varied UGT1A1 mRNA levels in different combined genotypes of A(TA)_{5–8}TAA and −3156G>A reveals that the lower UGT1A1 mRNA levels in the −3156A/A group may be due to a complete linkage disequilibrium with TA7/7 (Fig. 5B). The complete linkage disequilibrium between TA7 and −3156A was also reported in a study using cultured human hepatocytes (Ramírez et al., 2006), UGT1A1 activity measured by
metabolism of irinotecan was lower in hepatocyte samples carrying both TA7/7 and −3156A/A genotypes. The SNP of −3297T>G has a moderate effect on UGT1A1 transcription. However, when combined genotypes of A(TA)nTA and −3297T>G are taken into consideration, the liver samples carrying genotypes of TA7/7 plus −3297G/G and TA6/7 plus −3297G/G have significantly lower UGT1A1 mRNA levels than the liver samples carrying genotypes of TA6/6 plus −3297T/T, TA6/6 plus −3297T/G, TA6/7 plus −3297G/G, and TA6/7 plus −3297T/G (p < 0.01 and p < 0.05, respectively) (Fig. 5A). These data suggest that in addition to the TA7 allele, the −3297G allele has an effect to further lower the UGT1A1 transcription level. The −3297G allele has been suggested to associate with poor metabolism of bilirubin by UGT1A1 in hyperbilirubinemia and Gilbert’s syndrome (Sugatani et al., 2002; Maruo et al., 2004; Huang et al., 2008).

More importantly, the current study uses a population-based strategy to examine the influence of these genetic polymorphisms on UGT1A1 transcription mediated by the transcription factors, CAR, PXR, GR, HNF1α, and HNF4α. Without consideration of the genetic polymorphisms, mRNA levels of UGT1A1 are significantly correlated with mRNA levels of CAR, PXR, GR, HNF1α, and HNF4α in the liver samples examined (p < 0.001) (Table 1). However, when the genetic polymorphisms are taken into consideration, significant levels of mRNA correlation between UGT1A1 and its transcription factors, CAR, PXR, and GR, but not HNF1α and HNF4α, are changed in different genotype groups (Table 3). Significant correlation of mRNA levels between UGT1A1 and CAR does not exist in the samples carrying TA7/7 (p = 0.394) and −3156A/A (p = 0.304). Correlation of mRNA levels between UGT1A1 and PXR is not significant in the samples carrying TA7/7 (p = 0.153) and −3156A/A (p = 0.108). In addition, significant correlations between UGT1A1 and GR are lost in samples with TA7/7 (p = 0.409), TA6/7 (p = 0.074), −3156A/A (p = 0.144), and −3297G/G (p = 0.132). These genetic polymorphisms do not affect correlations between UGT1A1 and HNF1α or HNF4α (p < 0.01). These data indicate that the polymorphism of A(TA)nTA has a strong influence on CAR-, PXR-, and GR-mediated UGT1A1 transcription but not on HNF1α- and HNF4α-mediated transcription. The mRNA levels of UGT1A1 in liver samples with TA7/7 are much less dependent on the mRNA levels of CAR, PXR, or GR in liver samples with TA7/6 and TA6/6. Although −3156A/A also shows a strong influence on CAR-, PXR-, or GR-mediated UGT1A1 transcription, we cannot exclude the possibility of complete linkage disequilibrium between TA7/7 and −3156A/A.

The SNP of −3297T>G seems to have a limited influence on the transcription factor-mediated UGT1A1 transcription; however, when the combined genotypes between A(TA)nTA and −3297T>G are taken into consideration, clear patterns are observed (Fig. 6). Significant levels of mRNA correlation between UGT1A1 and CAR, PXR, or GR decrease in an order in the liver samples with combined genotypes of TA6/6 plus −3297T/T or −3297T/G or −3297G/G > TA6/7 plus −3297T/G > TA6/7 plus −3297G/G > TA7/7 plus −3297G/G. The influence of genetic polymorphisms on significant levels of correlation is much less between UGT1A1 and HNF1α or HNF4α. Using transfection assays, Sugatani et al. (2008) demonstrated that transcriptional activation of the UGT1A1 promoter by CAR, PXR, and GR ligands was reduced by both the A(TA)nTA and −3297T>G polymorphisms, but the A(TA)nTA polymorphism had a stronger influence than −3297T>G. A construct carrying both TA7 and −3297G gave the lowest induction by the nuclear receptor ligands compared with any other combinations of these two polymorphisms. Data from both the transfection assays (Sugatani et al., 2008) and liver population in the current study suggest that the genetic polymorphisms of A(TA)nTA and −3297T>G influence CAR-, PXR-, and GR-mediated activation of UGT1A1 transcription.

In conclusion, the current study demonstrates that the genetic polymorphisms of A(TA)nTA in the RNA Pol II core promoter and −3297T>G in the upstream enhancer element of the UGT1A1 promoter have a combined effect on UGT1A1 transcription. These polymorphisms also influence activation of UGT1A1 transcription by the transcription factors, CAR, PXR, and GR, which bind to the −3297T>G site. Both A(TA)nTA and −3297T>G are common in the general American population with the frequency of homozygous TA7 plus −3297G as high as 7 to 10% (Innocenti et al., 2002). Detection of these polymorphisms is important for identification of hyperbilirubinemia and prediction of UGT1A1-mediated drug metabolism and toxicity.

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References
Auerová S, Furihata T, Kobayashi K, and Chiba K (2007) Hepatocyte nuclear factor 1α and 4α are factors involved in interindividual variability in the expression of UGT1A6 and UGT1A9 but not UGT1A1, UGT1A3, and UGT1A4 mRNA in human livers. *Drug Metab Pharmacokinet* 22:358–364.


Address correspondence to: Dr. Xiao-bo Zhong, Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. E-mail: xzhong@kumc.edu