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, Pregnane X Receptor, or Glucocorticoid Receptor in Human Liver^[S]

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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) 1 α , and HNF4 α . The purpose of this study was to determine whether the genetic polymorphisms in the RNA polymerase II core promoter and the 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 *UGT1A1* TATA box and a single nucleotide polymorphism of -3279T>G in PBREM were genotyped in 98 human liver samples. Relative mRNA levels of CAR, PXR, GR, HNF1 α , HNF4 α , and UGT1A1 were quantified by a multiplex branched DNA technique. Correlations of mRNA levels between UGT1A1 and the transcrip-

tion factors were established in liver samples with different combined genetic polymorphisms. Correlation of mRNA levels between UGT1A1 and CAR, PXR, or GR, but not HNF1 α or HNF4 α , 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 TA6/7 plus -3279T/G and other genotypes containing TA6/6. In conclusion, the combined genotypes containing A(TA)₇TAA and -3279G decrease UGT1A transcription mediated by CAR, PXR, or GR but not by HNF1 α or HNF4 α .

Regulation of gene transcription by transcription factors is usually gene-specific and is governed by RNA polymerase II (Pol II) transcription machinery. DNA sequences in both the Pol II core promoter of a gene as well as enhancer elements of the gene are critical for increasing gene transcription by transcription factors. The Pol II core promoter is a short DNA sequence spanning approximately ± 40 base pairs around a transcriptional start site (TSS). It serves as a platform for the assembly of general transcription factors and the Pol II com-

plex to initiate transcription. A typical Pol II core promoter may contain one or several key elements, including a TATA box, initiator, downstream promoter element (DPE), and transcription factor IIA and IIB recognition elements (Butler and Kadonaga, 2002; Juven-Gershon et al., 2008). An enhancer element is a short region of DNA that binds transcription factors to increase transcription of target genes. After binding to an enhancer, transcription factors normally recruit coactivator complexes (such as chromatin-remodeling enzymes) to modify nucleosomes around the target TSS to gain access to the Pol II complex. Transcription factors can also interact with mediators for recruitment of general transcription factor II D for TSS recognition and for full assembly of the general transcription machinery on the Pol II core promoter elements (Black et al., 2006). An enhancer element can be located either immediately adjacent to a core promoter or more than 100 kilobases away (Carter et al., 2002; Zhu et al., 2007). Both

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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; PGC1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; PCR, polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LD, linkage disequilibrium.

FIG. 1. A, DNA sequence of the *UGT1A1* RNA polymerase II core promoter. A dinucleotide polymorphism of A(TA)₅₋₈TAA is located in the TATA box. B, locations of four SNPs in the *UGT1A1* upstream promoter. TATA box, TATAA sequence; DR1, DR3, DR4, nuclear receptor response motif with different nucleotide space; GRE, glucocorticoid response element; XRE, xenobiotic response element.

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)₅₋₈TAA in the TATA box is common in all ethnic populations, with A(TA)₆TAA as the major allele (Beutler et al., 1998). Minor alleles with higher TA repeat copy numbers, A(TA)₇TAA and A(TA)₈TAA, have a lower affinity to bind 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., 2002) 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, GR, or aryl hydrocarbon receptor ligands was reduced in a construct containing both A(TA)₇TAA 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* transcrip-

tion 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 α and HNF4 α 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 α (PGC1 α) 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 α (NM_000545), and HNF4 α (Z49825). PGC1 α (NM_013261) was selected 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 1

Distribution and correlation of gene transcription levels between *UGT1A1* and the transcription factors, *CAR*, *PXR*, *GR*, *HNF1 α* , and *HNF4 α*

Gene Name	Relative mRNA Levels				Mean after Log Transformation ^a	S.D. after Log Transformation	Pearson's Correlation ^b	
	Highest	Lowest	Mean	S.D.			<i>r</i>	<i>p</i>
% normalized with GADPH								
UGT1A1	84.7	1.7	20.8	17.9	1.180	0.352		
CAR	94.4	0.3	16.7	17.4	0.975	0.546	0.695	<0.001
PXR	51.5	0.3	12.6	10.4	0.930	0.437	0.602	<0.001
GR	40.7	3.7	10.4	6.0	0.967	0.191	0.398	<0.001
HNF1α	8.4	0.3	2.4	1.3	0.323	0.234	0.558	<0.001
HNF4α	134	6.9	35.2	19.8	1.493	0.216	0.565	<0.001
PGC1α	58.0	0.7	7.8	8.6	1.457	0.304	0.095	0.343

^a Log transformation: $X = \log_{10} X_r$, X_r , relative mRNA level after normalization with *GADPH*.^b 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 μ g of human liver total RNA was incubated overnight at 53°C with gene-specific xMAP 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*-phycoerythrin at room temperature for 30 min. Streptavidin-conjugated *R*-phycoerythrin 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 *GADPH* and expressed as a ratio of *GADPH* 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 GoTaq 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 Presequencing 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*, *HNF1 α* , and *HNF4 α* . Table 1 summarizes the highest, lowest, mean, and S.D. of mRNA

levels of *UGT1A1* and the transcription factors, *CAR*, *PXR*, *GR*, *HNF1 α* , and *HNF4 α* , 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*, *HNF1 α* , and *HNF4 α* . *PGC1 α* , 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 *PGC1 α* , 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 TA6/7 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 Cau-

FIG. 2. Distribution of the *UGT1A1* mRNA levels in the study population. The relative *UGT1A1* mRNA levels are presented as $X_i = (X - \text{mean})/\text{S.D.}$ When $X_i = 0$; X is the mean. When $X_i = 1$, X is 1 S.D. above the mean; when $X_i = -1$, X is 1 S.D. below the mean.

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.

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-AAAAAAAAAAAAAAAAAAAA-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

TABLE 2
Influence on the *UGT1A1* mRNA levels by the A(TA)₅₋₈TAA polymorphism with adjustment of the confounder factors

Factor	Regression Coefficient Estimate	S.E.	t Value	p Value
(Intercept)	-0.33	0.67	-0.49	0.62
Gender: male vs. female	-0.14	0.22	-0.65	0.51
Age	0	0.01	0.06	0.95
Ethnicity: AA vs. A	-0.14	0.67	-0.22	0.83
Ethnicity: C vs. A	-0.02	0.59	-0.04	0.96
Ethnicity: H vs. A	0.13	0.65	0.21	0.84
Death: AA vs. A	1.14	1.04	1.10	0.27
Death: CVA vs. A	0.54	0.33	1.64	0.10
Death: HT vs. A	0.39	0.37	1.05	0.29
Death: MI vs. A	-0.14	0.65	-0.23	0.82
Death: MVA vs. A	-0.04	0.74	-0.06	0.95
Smoking: yes vs. no	0.09	0.22	0.40	0.69
Alcohol: yes vs. no	0.16	0.22	0.70	0.48
Genotypes				
TA6/7 vs. TA6/6	0.03	0.23	-0.14	0.89
TA7/7 vs. TA6/6	-1.30	0.39	-3.30	<0.01

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. 4. Effects on the UGT1A1 gene transcription by the *UGT1A1* promoter polymorphisms. In each genotype group, a box represents mean and S.D.

(chip images in the top row of Fig. 3B). 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 Haplo-View 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)₇TAA, none of the possible confounding factors examined significantly influence UGT1A1 mRNA levels ($p > 0.05$). Samples with TA7/7 (including TA7/8) had significantly lower mRNA levels of UGT1A1 than samples with TA6/6 (including TA5/6) at $p < 0.01$ after adjusting for all potential factors, whereas samples with TA6/7 are not significantly different from samples with TA6/6 on mRNA levels of UGT1A1 after adjusting for all potential factors ($p = 0.89$).

Because no potential confounders are significantly associated with

FIG. 5. Effects on the UGT1A1 gene transcription by combined genotypes of A(TA)₅₋₈TAA and $-3279T>G$ (A) and A(TA)₅₋₈TAA and $-3156G>A$ (B).

TABLE 3

Correlation of mRNA levels between *UGT1A1* and CAR, PXR, GR, HNF1 α , HNF4 α , or PGC1 α in different genotype groups of A(TA)_{6/7}TAA, -3279T>G or -3156G>A

$p > 0.05$ in boldface.

<i>UGT1A1</i> Polymorphisms	Case I						Case II				Case III: PGC1 α	
	CAR		PXR		GR		HNF1 α		HNF4 α		r	p
	r	p	r	p	r	p	r	p	r	p		
A(TA) _{6/7} TAA												
TA6/6 ($n = 48$)	0.769	<0.001	0.637	<0.001	0.522	<0.001	0.567	<0.001	0.571	<0.001	0.118	0.421
TA6/7 ($n = 43$)	0.711	<0.001	0.572	<0.001	0.277	0.074	0.450	<0.01	0.501	<0.001	0.209	0.175
TA7/7 ($n = 7$)	0.385	0.394	0.601	0.153	0.374	0.409	0.910	<0.001	0.946	<0.01	0.663	0.104
-3156G>A												
G/G ($n = 44$)	0.719	<0.001	0.600	<0.001	0.535	<0.001	0.549	<0.001	0.540	<0.001	0.032	0.809
G/A ($n = 47$)	0.733	<0.001	0.634	<0.001	0.330	<0.05	0.478	<0.001	0.521	<0.001	0.164	0.266
A/A ($n = 7$)	0.508	0.304	0.718	0.108	0.671	0.144	0.910	<0.01	0.946	<0.01	0.620	0.189
-3279T>G												
T/T ($n = 31$)	0.727	<0.001	0.619	<0.001	0.603	<0.001	0.523	<0.01	0.500	<0.01	0.020	0.915
T/G ($n = 44$)	0.789	<0.001	0.620	<0.001	0.316	<0.05	0.516	<0.001	0.573	<0.001	0.283	0.061
G/G ($n = 23$)	0.518	<0.05	0.575	<0.01	0.324	0.132	0.640	<0.01	0.699	<0.001	0.100	0.657
Total	0.695	<0.001	0.602	<0.001	0.397	<0.001	0.558	<0.001	0.565	<0.001	0.095	0.342

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 -3156A/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 A(TA)₅₋₈TAA and -3279T>G or between A(TA)₅₋₈TAA 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 A(TA)₅₋₈TAA and -3279T>G or between A(TA)₅₋₈TAA and -3156G>A. Six different combined genotypes of A(TA)₅₋₈TAA 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 A(TA)₅₋₈TAA and -3156G>A were found in the study population (Fig. 5B). Only samples with TA7/7 plus -3156A/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 individ-

uals 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 A(TA)₅₋₈TAA, -3279T>G, or -3156G>A (Table 3). Three different cases were found. In case I, the association of gene transcription between *UGT1A1* and PXR, CAR, or GR decreased in genotype 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 HNF1 α or HNF4 α in all *UGT1A1* genotype groups. In case III, mRNA levels were not associated between *UGT1A1* and PGC1 α for all genotype groups.

Correlations of mRNA levels between *UGT1A1* and the transcription factors, CAR, PXR, GR, HNF1 α , or HNF4 α , were further examined in subgroups with various combined genotypes of A(TA)₅₋₈TAA and -3279 T>G (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 (***, $p < 0.001$); 2) association of gene transcription between *UGT1A1* and GR (Fig. 6C) were 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 (***, $p < 0.001$); and 3) association of gene transcription between *UGT1A1* and HNF4 α (Fig. 6D, similar between *UGT1A1* and HNF1 α ; 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, HNF1 α , or HNF4 α , in

FIG. 6. Scatter plots of mRNA levels of UGT1A1 versus CAR (A), PXR (B), GR (C), or HNF4 α (D) in the study population with different combined genotypes of A(TA)₅₋₈TAA and -3279T>G.

a human liver sample population, which has a composition of Caucasians, African-Americans, Hispanics, and Asians similar to that of the general American population. The genetic polymorphisms examined include a TA copy number polymorphism in the TATA box of the *UGT1A1* RNA Pol II core promoter and an SNP (-3279 T>G) in the upstream enhancer element, referred to as the phenobarbital-responsive enhancer module. Three SNPs of -3156G>A, -2353C>T, and -997G>A in the *UGT1A1* promoter are also included as controls.

After adjusting for potential confounding factors, such as age, gender, ethnicity, smoking, drinking, and reasons of death, we have found that liver samples carrying TA7/7 have significantly lower UGT1A1 mRNA levels than liver samples with TA6/6 and TA6/7 ($p < 0.01$) (Fig. 4). Liver samples carrying -3156A/A have significantly lower UGT1A1 mRNA levels than liver samples with -3156G/G and -3156G/A ($p < 0.05$). Liver samples carrying -3279G/G have significantly lower UGT1A1 mRNA levels than

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)₅₋₈TAA 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 -3279T>G has a moderate effect on UGT1A1 transcription. However, when combined genotypes of A(TA)₅₋₈TAA and -3279T>G are taken into consideration, the liver samples carrying genotypes of TA7/7 plus -3279G/G and TA6/7 plus -3279G/G have significantly lower UGT1A1 mRNA levels than the liver samples carrying genotypes of TA6/6 plus -3279T/T, TA6/6 plus -3279T/G, TA6/6 plus -3279G/G, and TA6/7 plus -3279T/G ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 5A). These data suggest that in addition to the TA7 allele, the -3279G allele has an effect to further lower the UGT1A1 transcription level. The -3279G 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, HNF1 α , and HNF4 α . Without consideration of the genetic polymorphisms, mRNA levels of UGT1A1 are significantly correlated with mRNA levels of CAR, PXR, GR, HNF1 α , and HNF4 α 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 HNF1 α and HNF4 α , 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 ($p = 0.409$), TA7/6 ($p = 0.074$), -3156A/A ($p = 0.144$), and -3279G/G ($p = 0.132$). These genetic polymorphisms do not affect correlations between UGT1A1 and HNF1 α or HNF4 α ($p < 0.01$). These data indicate that the polymorphism of A(TA)₅₋₈TAA has a strong influence on CAR-, PXR-, and GR-mediated UGT1A1 transcription but not on HNF1 α - and HNF4 α -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 -3156G/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 -3279T>G seems to have a limited influence on the transcription factor-mediated UGT1A1 transcription; however, when the combined genotypes between A(TA)₅₋₈TAA and -3279T>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 -3279T/T or -3279T/G or -3279G/G > TA6/7 plus -3279T/G > TA6/7 plus -3279G/G > TA7/7 plus -3279G/G. The influence of genetic polymorphisms on significant levels of correlation is much less between UGT1A1 and HNF1 α or HNF4 α . 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)₅₋₈TAA and -3279T>G polymorphisms, but the A(TA)₅₋₈TAA polymorphism had a stronger influence than -3279T>G. A construct carrying both TA7 and -3279G 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)₅₋₈TAA and -3279T>G influence CAR-, PXR-, and GR-mediated activation of UGT1A1 transcription.

In conclusion, the current study demonstrates that the genetic polymorphisms of A(TA)₅₋₈TAA in the RNA Pol II core promoter and -3279T>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 -3279T>G site. Both A(TA)₅₋₈TAA and -3279T>G are common in the general American population with the frequency of homozygous TA7 plus -3279G 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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