Table 1 Summary of *OCT1* and *OCT2* gene polymorphisms

Gene	Location	Position[a]	Allele[a]	Nucleotide sequence	Amino acid substitution	Allelic frequency (95% CI)	
						Responders (n = 24)	Non-responders (n = 9)
OCT1	Exon 1	123	C	tcttCctgg	41Phe > Leu	0.98 (0.94–1.02)	1.000
			G	tcttGctgg		0.02 (–0.02–0.06)	0.000
		156	T	agagTcctg	Ser52	0.58 (0.44–0.72)	0.44 (0.21–0.67)
			C	agagCcctg		0.42 (0.28–0.56)	0.56 (0.33–0.79)
		243	C	cgggCgagg	Gly81	1.000	0.94 (0.84–1.05)
			T	cgggTgagg		0.000	0.06 (–0.05–0.16)
		350	C	ctgcCgctg	117Pro > Leu	1.000	0.94 (0.84–1.05)
			T	ctgcTgctg		0.000	0.06 (–0.05–0.16)
	Intron 1	–43	T	atggTtctg	–	0.42 (0.28–0.56)	0.33 (0.12–0.55)
			G	atggGtctg		0.58 (0.44–0.72)	0.67 (0.45–0.89)
	Exon 2	480	C	tcttCtttg	160Phe > Leu	0.88 (0.78–0.97)	0.83 (0.66–1.01)
			G	tcttGtttg		0.13 (0.03–0.22)	0.17 (–0.01–0.34)
	Exon 6	1022	C	acgcCgcgc	341 Pro > Leu	0.81 (0.70–0.92)	0.89 (0.74–1.03)
			T	acgcTgcgc		0.19 (0.08–0.30)	0.11 (–0.03–0.26)
	Exon 7	1222	A	ggccAtgtc	408Met > Val	0.19 (0.08–0.30)	0.28 (0.07–0.49)
			G	ggccGtgtc		0.81 (0.70–0.92)	0.72 (0.52–0.93)
	Inron 7	+8	Deletion	(ggtaagtt)0		0.81 (0.70–0.92)	0.72 (0.52–0.93)
				(ggtaagtt)1		0.19 (0.08–0.30)	0.28 (0.07–0.49)
	Intron 10	+26	C	actcCgagg		0.98 (0.94–1.02)	1.000
			T	actcTgagg		0.02 (–0.02–0.06)	0.000
		–21	C	ccaaCttt		0.46 (0.32–0.60)	0.39 (0.16–0.61)
			T	ccaaTttt		0.54 (0.40–0.68)	0.61 (0.39–0.84)
OCT2	Exon 3	602	C	tataCgtgg	201Thr > Met	0.98 (0.94–1.02)	0.94 (0.84–1.05)
			T	tataTgtgg		0.02 (–0.02–0.06)	0.06 (–0.05–0.16)
	Exon 4	808	G	agttGctct	270Ala > Ser	0.92 (0.88–0.96)	0.94 (0.84–1.05)
			T	agttTctct		0.08 (0.04–0.12)	0.06 (–0.05–0.16)

[a] Position is relative to the ATG start site, and the reference allele for each gene was obtained from the GenBank accession numbers AL353625 for OCT1 and AL162582 for OCT2

408Met > Val (1222A/1222G), and homozygotes for 408Val (1222G/1222G) was 0.69 ± 0.43, 0.92 ± 0.53, and 1.01 ± 0.66, respectively. Although the hepatic expression of OCT1 tended to be lower in livers with the 408Met (1222A) variant, the differences did not reach the level of significance. In the –43T > G variant, the mean OCT1 expression level in –43T/T (n = 18), –43T/G (n = 8), and –43G/G (n = 10) samples (all harbored the 1222G/1222G allele) was 1.01 ± 0.70, 1.04 ± 0.34, and 1.46 ± 0.53, respectively.

Table 2 Stepwise discriminant functional analysis of the efficacy of metformin

Variable	Coefficient	χ^2 value	P
Age	0.09	5.59	0.05
BMI	0.23		
Treatment with lipid-lowering agents	2.25		
–43T > G (intron 1)	–2.35		
408Met > Val (exon 7)	2.51		

Predictive accuracy = 55.5%

Discussion

In this study we first analyzed mutations in *OCT1* and *OCT2* and then examined the association between polymorphisms in these two genes and the efficacy of metformin, because in vitro studies have indicated that OCT1 and OCT2 are responsible, respectively, for the hepatic and renal transport of metformin (Kimura et al. 2005; Wang et al. 2002, 2003). In contrast to studies in vitro and with animals, there are no data from human studies on the contribution of these polymorphisms to the phenotypes of metformin.

In the *OCT1* gene, all non-synonymous variants except 41Phe > Leu and 117Pro > Leu have already been identified in some racial populations, with a frequency of 0.005–0.81 (Kerb et al. 2002; Shu et al. 2003). The 41Phe > Leu and 117Pro > Leu allele frequencies were relatively low (0.004), and they have already been reported in a Japanese population (Itoda et al. 2004). Recent expression studies have indicated that 341Pro > Leu had decreased ability to transport test compounds, while 160Phe > Leu and 408Met > Val were unchanged (Kerb et al. 2002; Sakata et al. 2003;

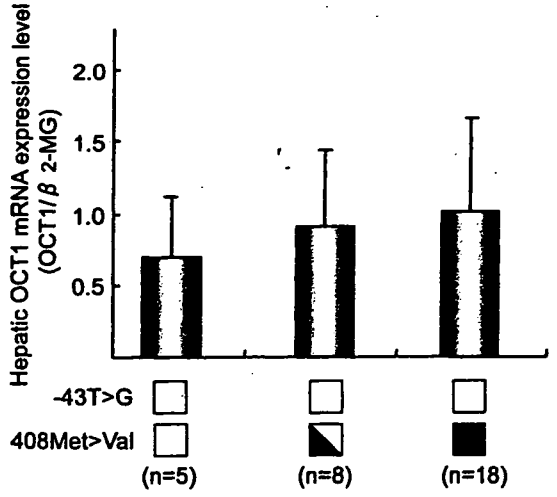

Fig. 2 Hepatic OCT1 mRNA expression levels with regard to the 408Met > Val (1222A > G) variant. Among 58 samples, 31, which were homozygotes for the –43T variant (–43T/T), were analyzed. *Open squares*, *partially filled squares* and *closed squares* correspond to patients homozygous for the 408Met (1222A) allele and heterozygous and homozygous for the 408Val (1222G) allele

Shu et al. 2003). Interestingly, the 341Pro > Leu variant was observed in Asian and African American populations but not in Caucasians (Shu et al. 2003); however, there was no difference in the allele frequency of 341Pro > Leu between responders and non-responders to metformin therapy in this study.

In contrast to those in the *OCT1* gene, it appears that the number of non-synonymous variants in the *OCT2* gene and their allelic frequencies were lower than in other known drug transporter genes such as *MDR1*, *MRP1*, *MRP2*, and *OATP-C* (Nishizato et al. 2003). These observations are consistent with the finding of a lower frequency of non-synonymous variants in ethnically diverse genomic DNA samples (Leabman et al. 2002). Recent population-genetic analysis has demonstrated that selection has acted against amino acid changes in OCT2 (Leabman et al. 2002), suggesting that OCT2 is relatively intolerant of non-synonymous changes. In general, the less frequent non-synonymous variants resulted in more significant and deleterious functional changes. However, the 270Ala > Ser variant was reported to exhibit subtle functional differences from the reference form of OCT2 (Leabman et al. 2002).

Although there were no remarkable differences in the prevalence of any mutation sites between responders and non-responders, we next carried out discriminant functional analysis including not only genetic polymorphisms but also the patients' background. As shown in Table 2, age, BMI and treatment with lipid-lowering agents were demonstrated as positive predictors of metformin efficacy. These observations are partially in agreement with the findings by Knowler et al. (2002), that metformin was less effective in subjects with lower BMI or a lower fasting plasma glucose concentration. BMI > 25 kg/m^2 is defined as obesity in Japan; 66.7% of responders and 44.4% of non-responders were obese in this study. Although the precise mechanism is unknown, these data suggest that metformin is more effective in the case of obesity-induced insulin resistance that is higher fasting plasma glucose. The contribution of lipid-lowering agents was somewhat unexpected, because metformin therapy has been reported to improve both glycemic control and lipid concentrations (i.e., plasma total and low-density lipoprotein cholesterol and triglyceride) in patients with non-insulin-dependent diabetes mellitus (DeFronzo and Goodman 1995). However, in our study, 12 responders and two non-responders were treated with lipid-lowering agents, and most of these patients (11/12 responders and 1/2 non-responders) used HMG-CoA reductase inhibitors (statins). Several studies have shown that low-density lipoprotein (LDL) size rather than plasma LDL level is more correlated with insulin resistance and eventual progression of coronary heart disease (Rizzo and Berneis 2006). Although the efficacy of modifying LDL size is different among agents (fluvastatin and atorvastatin seem to be much more effective agents than pravastatin and simvastatin), statins moderately lower all LDL subclasses, and, somehow, this process seems to make metformin more effective.

Since –43T > G and 408Met > Val (1222A > G) variants were identified as negative and positive predictors, respectively, for the clinical effectiveness of metformin, we evaluated the functional significance of the latter non-synonymous variant in the expression of OCT1 mRNA, using human liver samples. Our findings indicate that samples with the 408Met (1222A) allele tended to be associated with a reduced expression level, as compared with those without the 408Met allele; however, the difference did not reach significance. A recent study using site-directed mutagenesis has indicated that point mutations in the predicted ninth transmembrane domain such as 1222A > G (408Met > Val) do not lead to functional changes (Kerb et al. 2002). We also measured OCT1 mRNA expression with regard to the non-coding –43T > G variant; however, no significant effect was observed. In the present study, the predicted accuracy is still insufficient for its clinical application (i.e., 55.5%). Thus, if these observations are taken into consideration, the contribution of polymorphisms in

OCT1 and *OCT2* genes to metformin efficacy may not be as significant as our expectations had led us to believe. However, since a non-synonymous variant 408Met > Val is often observed simultaneously with other non-synonymous variants (Shu et al. 2003), further in vitro and in vivo studies with regard to the haplotypic consideration, including the non-coding region, are needed to elucidate the functional properties of the variants identified in this study.

While data from only 24 responders and nine non-responders were used, this preliminary investigation is the first study addressing the genotype–phenotype relationship of OCTs in the efficacy of metformin. However, obviously, the small number of patients is a drawback in our study. For example, co-medication of other anti-hyperglycemic drugs in both groups made it difficult for us to judge whether the decreases in HbA_{1c} levels in the responders are attributable to the metformin effect. Clearly, definition of the clinical cut-off point is also essential to divide patients into the two groups correctly. In order to overcome these problems, it is clear that the results in this study should be confirmed in a population study involving large numbers of patients. Nevertheless, this report provides for the possibility of OCTs' functions in humans.

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A Combinatorial Haplotype of the UDP-Glucuronosyltransferase 1A1 Gene (*60-*IB) Increases Total Bilirubin Concentrations in Japanese Volunteers

To the Editor:

UDP-glucuronosyltransferases (UGTs) are a family of enzymes that glucuronidate many endogenous and exogenous substrates (*1*). Of the *UGT1A* gene isoforms, *UGT1A1* is primarily responsible for glucuronidation of bilirubin (*1*). In east Asians, 2 well-known genetic variants, $A(TA)_6TAA>A(TA)_7TAA$ (allele **28*, reduced transcription) and G71R (211G>A, allele **6*, reduced activity), are causative factors for increased plasma bilirubin concentrations in Gilbert syndrome (*1*). The **28* allele is almost always linked to the **60* allele (-3279T>G), with reduced in vitro transcription (*2*).

In a previous study (*2*) in which we divided *UGT1A1* into 2 haplotype blocks (the 5′-flanking region and exon 1 in block 1 and common exons 2 to 5 in block 2), **60* and **IB* (perfectly linked 1813C>T, 1941C>G, and 2042C>G in the 3′-untranslated region in Japanese persons) showed increased total bilirubin concentrations in non-**28* patients. Because of the small number of patients, however, it was not clear whether bilirubin concentrations were affected by **60* and **IB* acting independently or cooperatively when they were on the same chromosome. To clarify this point, we reinvestigated the associations between the *UGT1A1* haplotypes and total bilirubin concentrations in 554 healthy Japanese volunteers. The ethical review boards of the participating institutions approved this study, and informed consent was obtained from all participants.

For genotyping of **60*, **28*, **6*, and **IB* marker variations, DNA was extracted from Epstein-Barr-virus–transformed lymphoblastoid cells. The genotyping methods for the **60*, **6*, and **IB* alleles were described previously (*3*, *4*). For **IB*, 1941C>G was genotyped (*3*). For **28*, −364C>T, which is perfectly linked with the **28* allele in Japanese persons (*2*), was used as a surrogate polymorphism, as described in Fig. 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol53/issue2, which also shows the allele frequencies of the variations. The diplotype configuration (combination of haplotypes) for each volunteer was inferred by an expectation-maximization–based program, LDSUPPORT, as

described previously (2, 5). Diplotype configurations of the 521 volunteers without heterozygous *60 and *IB were obtained at 1.00 probability. Previously, we reported that *UGT1A3* 17A>G is linked with both *60 and *IB (5), and *IB is not linked with *28 and *6 (2). When *UGT1A3* 17A>G was included, all the diplotype configurations were inferred with >0.95 probability.

To differentiate between allele and haplotype names, haplotypes are indicated by the # symbol plus the representative allele name. The haplotypes without marker variations were designated #1 for Block 1 and #IA for Block 2 (2). Note that the *28 allele was perfectly linked with the *60 allele, but only half of the *60 allele, approximately, was linked with the *28 allele. Thus, the #28 haplotype harbors both *28 and *60 alleles, whereas the #60 haplotype harbors only the *60 allele, as reported previously (2). The most frequent haplotype was #1-#IA (frequency, 0.545), followed by, in order, #6-#IA (0.171), #28-#IA (0.107), #60-#IA (0.079), #1-#IB (0.060), and #60-#IB (0.038).

We investigated the association of *UGT1A1* haplotypes with total bilirubin concentrations (Fig. 1). *P* values <0.05 were considered significant. We used the Kruskal–Wallis test ($P < 0.0001$) for statistical analysis of the differences in bilirubin concentrations among all diplotypes, followed by the nonparametric Dunnett multiple comparison test. Significant increases in bilirubin concentrations were observed in the #6-#IA/#28-#IA, #6-#IA/#6-#IA, #6-#IA/#60-#IB, #60-#IA/#28-#IA, and #28-#IA/#28-#IA volunteers compared with the #1-#IA/#1-#IA volunteers. An increasing trend (statistically not significant) in bilirubin concentrations (2.4-fold increase) was seen in the two #60-#IB/#28-#IA volunteers compared with the #1-#1A/#1-#1A volunteers. Significant increases in bilirubin concentrations have already been reported for #6 (#6-#IA in this study) and #28 (#28-#IA) (1). Note that the median of total bilirubin values was not increased in the heterozygotes of #6-#IA (#1-#IA/#6-#IA) and #28-#IA (#1-#IA/#28-#IA; Fig. 1).

We next analyzed the additive effects of #60-#IB and #60-#IA on #6-#IA and #28-#IA, respectively. A significant increasing effect of #60-#IB on #6-#IA was observed for #6-#IA/#60-#IB compared with #1-#IA/#6-#IA ($P = 0.0093$; Mann–Whitney *U*-test). However, when #60-#IA/#28-#IA was compared with #1-#IA/#28-#IA, the effect of #60-#IA was not statistically significant ($P = 0.0513$).

This study shows that either #60 or #IB alone has a slight effect on total bilirubin concentrations. The presence of both #60 and #IB on the same DNA strand (#60-#IB), however, significantly increased bilirubin concentrations when present with #6-#IA on the other chromosome. Thus, at least in the Japanese population, #60 and #IB marker variations should also be incorporated into the *UGT1A1* genotyping in addition to #6 and #28 markers.

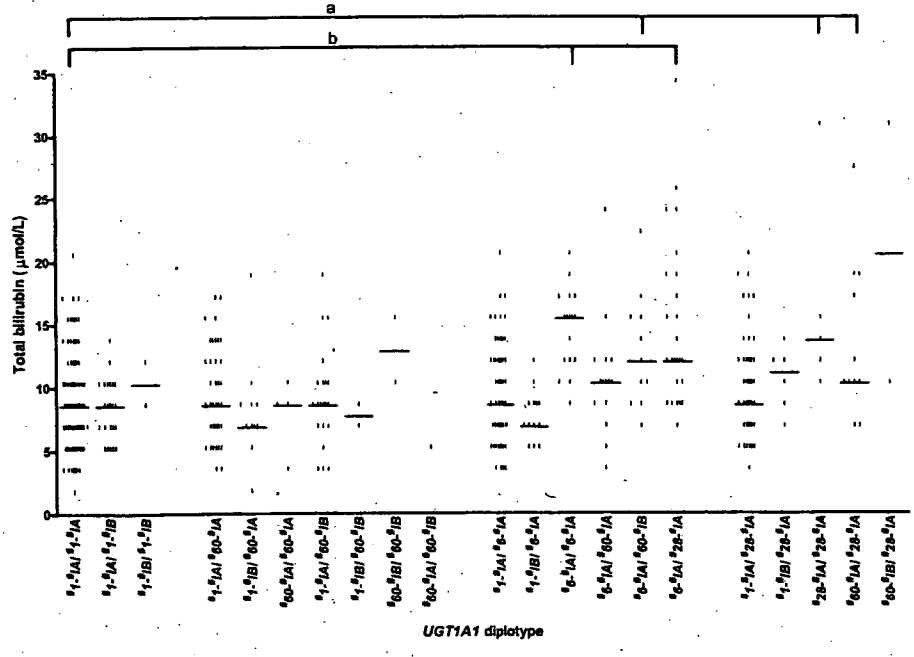

Fig. 1. Association of the *UGT1A1* diplotypes with increased total bilirubin concentrations in 554 Japanese healthy volunteers.

Haplotypes are shown with # plus representative allele name. Note that haplotype #28 harbors both *28 and *60 alleles (see Fig. 1 in the online Data Supplement). Each *point* represents 1 volunteer, and the median is indicated by a *horizontal bar*. The Kruskal–Wallis test for the 21 diplotypes yielded a *P* value of <0.0001. Significant increases in bilirubin concentrations were detected in #6-#IA/#28-#IA ($P < 0.0001$), #6-#IA/#6-#IA ($P < 0.0001$), #6-#IA/#60-#IB ($P = 0.0133$), #60-#IA/#28-#IA ($P = 0.0186$), #28-#IA/#28-#IA ($P = 0.0213$), compared with #1-#IA/#1-#IA (nonparametric Dunnett multiple comparison test). *a*, $P < 0.05$; *b*, $P < 0.0001$.

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Note

Hepatocyte Nuclear Factor 1 Alpha and 4 Alpha are Factors Involved in Interindividual Variability in the Expression of UGT1A6 and UGT1A9 but not UGT1A1, UGT1A3 and UGT1A4 mRNA in Human Livers†

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: UDP-glucuronosyltransferases (UGTs) catalyze phase-II biotransformation reaction of a variety of substances. Among the UGT1A isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A9 are predominantly expressed in the liver. Interindividual variability in expression of these isoforms would cause interindividual differences in drug response, toxicity and cancer susceptibility. In the present study, we investigated the interindividual variability in UGT1A mRNA expression and whether hepatocyte nuclear factor 1α (HNF1α) and HNF4α were factors responsible for their variability in human livers. The amounts of UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, HNF1α and HNF4α mRNA in 18 human livers were measured by quantitative real-time polymerase chain reaction. The largest and smallest interindividual differences in expression levels were observed in UGT1A1 (8.6-fold) and UGT1A4 (2.5-fold) mRNA, respectively. The amounts of HNF1α and HNF4α mRNA were strongly correlated with the amount of UGT1A9 mRNA and moderately correlated with that of UGT1A6 mRNA, whereas no significant correlation was found with the amounts of UGT1A1, UGT1A3 and UGT1A4 mRNA. Our results suggest that HNF1α and HNF4α are the factors involved in the interindividual variability of UGT1A6 and UGT1A9 mRNA expression. Further studies of other transcription factors are needed to clarify the factor(s) determining the interindividual variations in UGT1A1, UGT1A3 and UGT1A4 mRNA expression.

Key words: UDP-glucuronosyltransferase; hepatocyte nuclear factor; gene regulation; interindividual variability; human liver

Introduction

UDP-glucuronosyltransferases (UGTs) catalyze the glucuronidation of many endogenous compounds, drugs and carcinogens, which is one of the important detoxification pathways.[1] On the basis of the homology of amino acid sequences, UGTs have been categorized into two families, UGT1 and UGT2.[2] UGT1A isoforms, encoded from a single gene with alternative promoters located on chromosome 2q37, have been characterized in humans.[2] Through a process of RNA splicing, nine UGT1A isoforms are generated with an individual first exon joined to identical exons 2 to 5.[3] Among the UGT1A isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A9 have been shown to be predominantly expressed in the liver,[4] where they contribute to more than half of total drug glucuronidation.[5]

It is likely that interindividual variability of hepatic

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UGT1A expression plays an important role in drug efficacy, xenobiotic toxicity and cancer susceptibility.[6] To date, there have been only limited reports showing interindividual variability in the hepatic expression of UGT1A mRNA. Congiu *et al.*[7] reported that interindividual variations in the amounts of UGT1A and UGT2B mRNA ranged from 5- to 15-fold in human livers and that the variations in UGT1A4, UGT2B4 and UGT2B7 mRNA levels were related to the degree of liver inflammation. Two other studies showed remarkable interindividual variations in UGT1A1 and UGT1A6 mRNA expression levels in human livers.[8,9] However, the molecular mechanism(s) involved in the interindividual difference in UGT1A mRNA expression has not been fully elucidated. Although it has been reported that genetic polymorphisms in the 5′-flanking region and environmental factors, such as drugs, tobacco and alcohol consumption, are associated with the interindividual differences in UGT1A1 and UGT1A6 mRNA expression levels,[8,9] these factors cannot entirely explain their interindividual variations.

Besides genetic and environmental factors, the interindividual variation in UGT1A mRNA expression may result from different expression levels of transcription factors controlling their expression between individuals. Recently, the proximal promoters of several human *UGT* genes have been studied and the transcription factors that regulate these promoters have been identified.[6,10-14] The results of these studies have shown that HNF1α and HNF4α, which are the major liver-enriched transcription factors,[15] can bind and activate *UGT1A* promoters. Potential binding sites for HNF1α were found in *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A9* and rat *Ugt1a6* gene promoters and their functions were characterized by luciferase assays and electrophoretic mobility shift assays.[6,11-14] In addition to HNF1α, HNF4α has been shown to activate and bind to the *UGT1A9* gene promoter.[13,14] Based on the results of these *in vitro* studies, it is reasonable to hypothesize that HNF1α and HNF4α are factors determining the hepatic expression of UGT1A mRNA *in vivo*. However, the significance of HNF1α and HNF4α in the expression of UGT1A mRNA in human livers has not been clarified.

In the present study, we investigated the interindividual variability in UGT1A mRNA expression and whether HNF1α and HNF4α were factors responsible for their variability in human livers. The expression levels of UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A9 mRNA in 18 human liver samples were quantified and their correlations with the amounts of HNF1α and HNF4α mRNA were examined.

Materials and Methods

Human liver samples: Japanese liver samples (n = 18) were obtained from National Cancer Hospital East (Kashiwa, Japan). The liver samples were part of tissues surrounding tumor areas that had been surgically resected from donors with hepatocarcinoma metastasis from colorectal or gastric cancer. The liver tissues were snap-frozen and stored in liquid nitrogen until use for RNA isolation. This study was approved by the Ethics Committee of Chiba University (Chiba, Japan).

RNA isolation and cDNA synthesis: Total RNA was isolated from liver tissues by using an SV Total Isolation System (Promega, Madison, WI) according to the manufacturer's instructions. The isolated total RNA was then treated with RNase-free DNase I (Takara, Shiga, Japan) to remove contaminating genomic DNA, and the integrity of each RNA sample was evaluated by determination of the ratio of 28S rRNA to 18S rRNA by formaldehyde-agarose gel electrophoresis. The cDNA was generated with a random hexamer by using Ready-to-Go™ RT-PCR Beads (GE Healthcare, Little Chalfont, UK).

Quantification of hepatic UGT1A, HNF1α and HNF4α mRNA expression levels: The expression levels of UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, HNF1α and HNF4α mRNA in human liver tissues were measured by quantitative real-time PCR carried out on an ABI PRISM® 7000 (Applied Biosystems, Foster city, NJ) and using TaqMan Gene Expression Assays specific for each gene (FAM/MGB Probe, Applied Biosystems). The PCR amplification was performed as described previously.[16] The expression levels of UGT1A, HNF1α and HNF4α mRNA were normalized by the average values of four housekeeping genes (cyclophilin, β-glucuronidase, acidic ribosomal protein and glyceraldehyde-3-phosphate dehydrogenase) based on previous reports.[17,18] The mRNA expression levels of genes are expressed in arbitrary units, with the lowest mRNA expression level of each gene being assigned to 1. The amount of mRNA was measured in duplicate, and each value presented is the mean from three independent measurements.

Statistical analysis: Correlations between the amounts of UGT1A mRNA and those of HNF1α and HNF4α mRNA were determined by univariated linear regression analysis. A p value <0.05 was considered statistically significant.

Results

Interindividual variability of UGT1A mRNA expression levels in human livers: The extents of interindividual variability of UGT1A mRNA expression were different among isoforms (**Fig. 1**). The highest to lowest extents of interindividual differences in expression levels were observed in UGT1A1 (8.6-fold), UGT1A3 (6.5-fold), UGT1A9 (5.1-fold), UGT1A6 (4.9-fold) and UGT1A4 (2.5-fold) mRNA in that order.

Interindividual variability of HNF1α and HNF4α

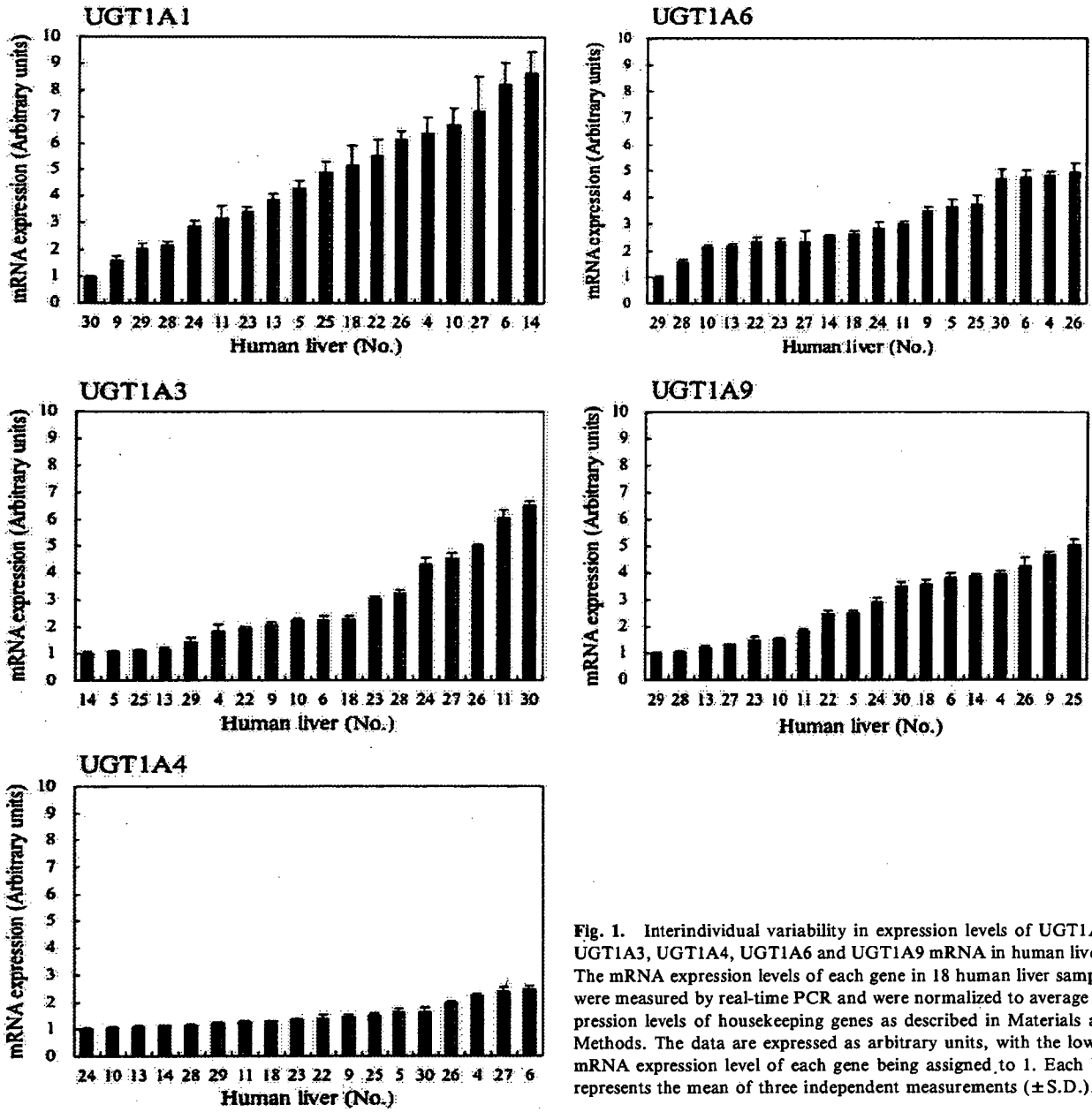

Fig. 1. Interindividual variability in expression levels of UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT1A9 mRNA in human livers. The mRNA expression levels of each gene in 18 human liver samples were measured by real-time PCR and were normalized to average expression levels of housekeeping genes as described in Materials and Methods. The data are expressed as arbitrary units, with the lowest mRNA expression level of each gene being assigned to 1. Each bar represents the mean of three independent measurements (±S.D.).

mRNA expression levels and their correlation in human livers: Among individuals, HNF1α and HNF4α mRNA expression levels varied 8.0- and 18-fold, respectively (**Fig. 2A**). The high correlation was observed between the expression levels of HNF1α and HNF4α mRNA ($r=0.70$, $p<0.005$) (**Fig. 2B**), being consistent with the results of the previous reports showing that HNF1α and HNF4α can bind to each other's promoter to positively regulate the expression of each other.[19-21]

Correlations between UGT1A mRNA and HNF1α mRNA expression levels: When compared among UGT1A isoforms, the strongest correlation was found between UGT1A9 mRNA and HNF1α mRNA expression levels ($r=0.79$, $p<0.0001$) (**Fig. 3**). The correlation between UGT1A6 mRNA and HNF1α mRNA expression levels was also statistically significant ($r=0.52$, $p<0.05$). In contrast, there was no significant correlation between UGT1A1, UGT1A3, UGT1A4 mRNA expression levels and HNF1α mRNA expression level.

Correlations between UGT1A mRNA and HNF4α mRNA expression levels: Similar to HNF1α, a significant correlation was found between UGT1A9 mRNA and HNF4α mRNA expression levels ($r=0.66$, $p<0.005$) (**Fig. 4**). A significant correlation was also

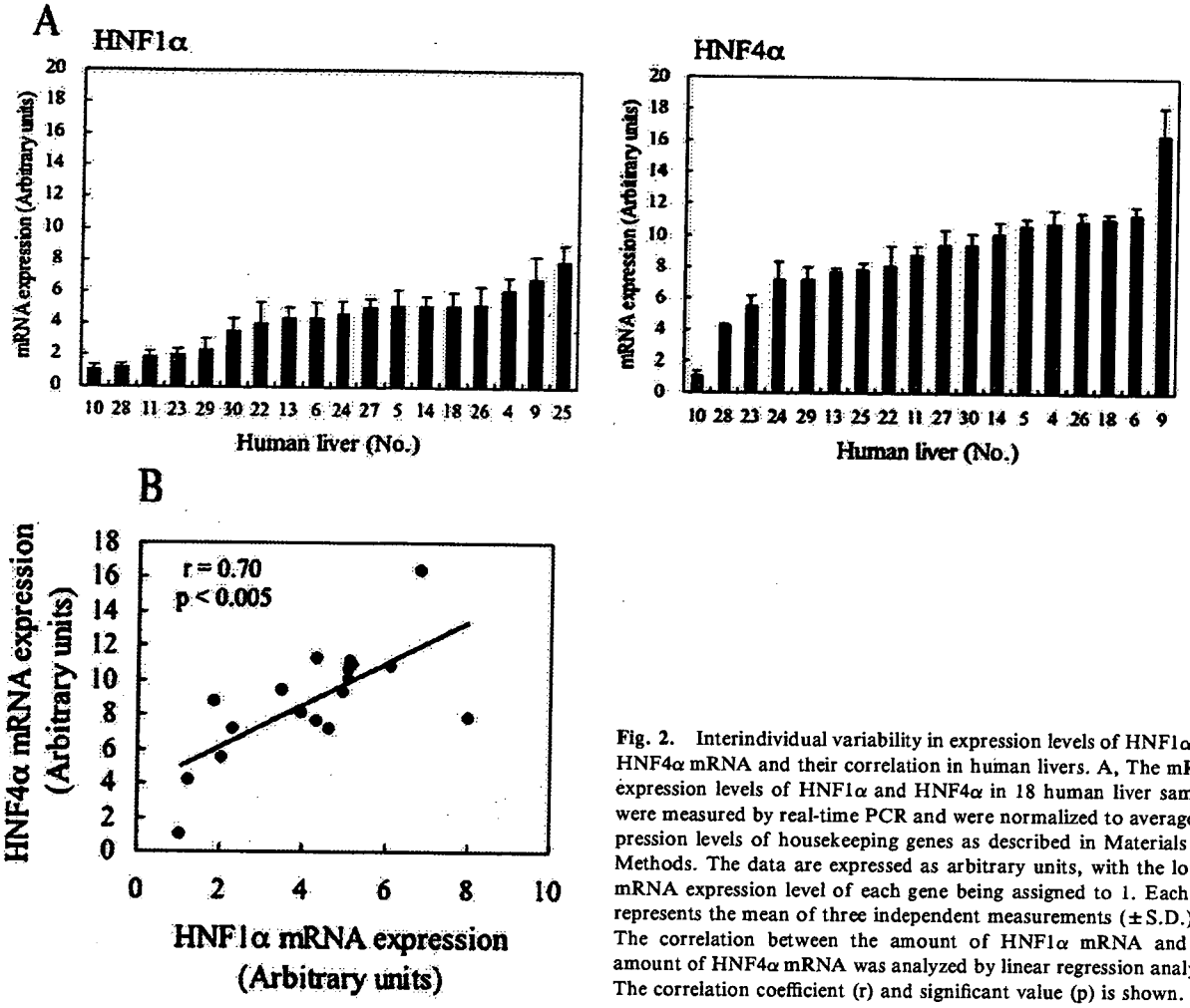

Fig. 2. Interindividual variability in expression levels of HNF1α and HNF4α mRNA and their correlation in human livers. A, The mRNA expression levels of HNF1α and HNF4α in 18 human liver samples were measured by real-time PCR and were normalized to average expression levels of housekeeping genes as described in Materials and Methods. The data are expressed as arbitrary units, with the lowest mRNA expression level of each gene being assigned to 1. Each bar represents the mean of three independent measurements (±S.D.). B, The correlation between the amount of HNF1α mRNA and the amount of HNF4α mRNA was analyzed by linear regression analysis. The correlation coefficient (r) and significant value (p) is shown.

found between UGT1A6 mRNA and HNF4α mRNA expression levels ($r=0.55$, $p<0.05$), whereas there was no significant correlation between UGT1A1, UGT1A3, UGT1A4 mRNA expression levels and HNF4α mRNA expression level.

Discussion

We hypothesized that HNF1α and HNF4α were factors determining the expression of UGT1A mRNA in the human liver. Our results showed that the amounts of HNF1α and HNF4α mRNA were highly correlated with the amount of UGT1A9 mRNA (**Figs. 3 and 4**), suggesting that both HNF1α and HNF4α are factors determining the hepatic UGT1A9 mRNA expression. These findings are consistent with the results of previous *in vitro* studies characterizing the *UGT1A9* promoter.[13,14] UGT1A9 is the only isoform of the *UGT1A7-10* gene cluster known to be expressed in the liver.[2] Recently, the functional binding sites of both HNF1α and HNF4α have been found in the *UGT1A9* gene promoter at positions −290 to −278 and −235 to −223, respectively.[14] Mutation of these binding sites dramatically reduced the ability of HNF1α and HNF4α to activate a UGT1A9 2kb reporter construct. In addition, Gardner-Stephen *et al.*[14] reported a synergistic role of HNF1α and HNF4α in regulation of the *UGT1A9* promoter. However, the identified HNF1α and HNF4α-binding elements in the *UGT1A9* promoter were not conserved in the *UGT1A7*, *UGT1A8* and *UGT1A10* promoters, which are not expressed in the liver.[14] Therefore, both HNF1α and HNF4α are thought to be important transcription factors contributing to unique hepatic expression of UGT1A9 amongst the *UGT1A7-10* gene cluster. Taken together, our results suggest that both HNF1α and HNF4α are the factors determining the constitutive expression of the *UGT1A9* gene and are factors involved in the interindividual variability in UGT1A9 mRNA expression in human livers.

In addition to the *UGT1A9* gene, our results suggest that HNF1α and HNF4α are also factors determining

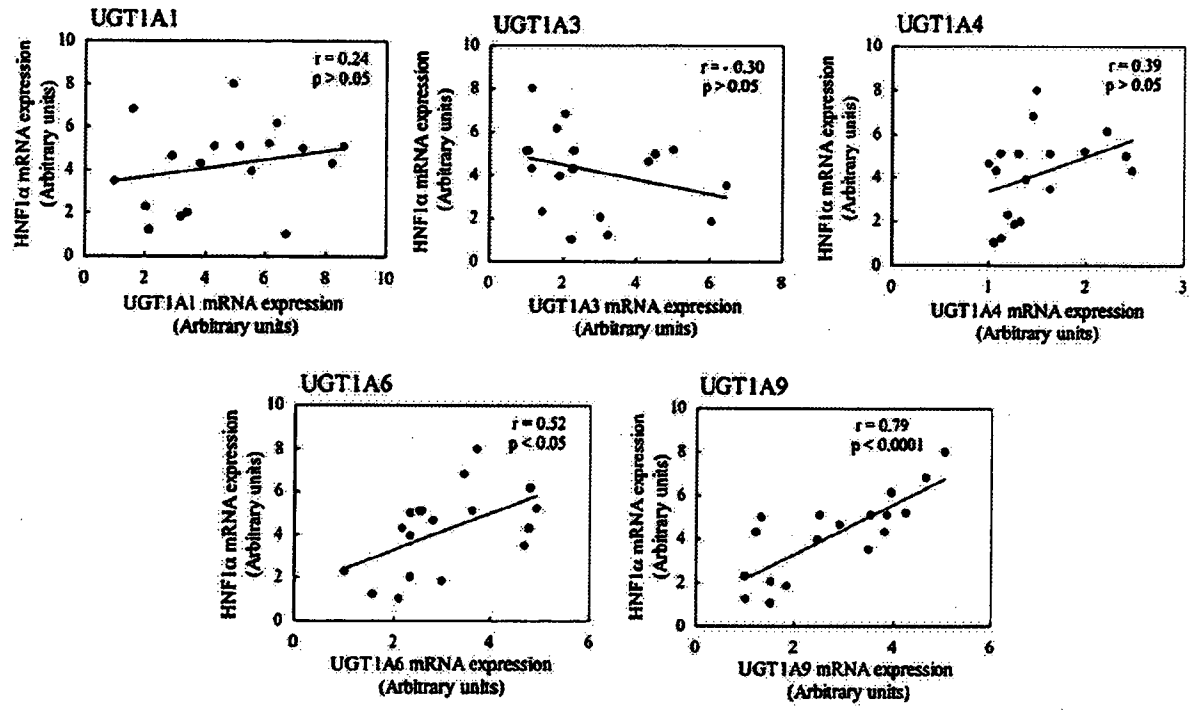

Fig. 3. Correlations between expression levels of UGT1A mRNA (UGT1A1, 1A3, 1A4, 1A6 and 1A9) and HNF1α mRNA in human livers. The correlations between the amounts of UGT1A mRNA and the amount of HNF1α mRNA were analyzed by linear regression analysis. The correlation coefficient (r) and significant value (p) for each correlation is shown.

the hepatic expression of UGT1A6 mRNA, although the strength of the correlation was not high (**Figs. 3 and 4**). To date, there has been no study on the role of HNF1α and HNF4α in hepatic expression of the human *UGT1A6* gene. However, it has been shown that HNF1α can stimulate activity of the P2 promoter of the rat *Ugt1a6* gene.[12] This HNF1α-binding site has also been conserved in the promoter of the human *UGT1A6* gene at positions −169 to −157 (GTTAAATATTAAT).[6] As for HNF4α, examination of the DNA sequence of the human *UGT1A6* promoter by using computer analysis revealed a possible HNF4α-binding site at positions −750 to −738 (TGTTCTTTGTACT) (http://www.gene-regulation.com).[22] Although further studies on the function of HNF1α and HNF4α in the human *UGT1A6* gene promoter are needed, our results suggest that constitutive expression of the *UGT1A6* gene in the human liver could be under the control of both HNF1α and HNF4α as in the case of the *UGT1A9* gene. Taken together, the results suggest that HNF1α and HNF4α are likely to be factors determining the interindividual difference in hepatic UGT1A6 mRNA expression.

In contrast to the *UGT1A6* and *UGT1A9* genes, the amounts of UGT1A1, UGT1A3 and UGT1A4 mRNA were not significantly correlated with those of HNF1α and HNF4α mRNA (**Figs. 3 and 4**). It is noteworthy that while all hepatic *UGT1A* genes possess an HNF1α -binding site in their proximal promoters,[6,11,12,14] their significance in the expression of *UGT1A* genes in the liver varies. Another example of this type of discrepancy has been reported in the *UGT2B15* gene. Although it was reported that the putative HNF1α-binding site was found in the *UGT2B15* promoter and that HNF1α was found to occupy the *UGT2B15* promoter in hepatocytes by using chromatin immunoprecipitation combined with promoter microarrays,[6,21] the amount of HNF1α mRNA did not correlate with the expression level of UGT2B15 mRNA.[23] These results suggest that even if there are functional HNF1α-binding sites, the contribution of these sites to gene expression in the liver is dependent on the individual *UGT* genes. These variations may be caused by larger regulatory networks of transcription factors occurring *in vivo*. In a chromosomal setting, HNF1α can cooperate with other transcription factors to form a regulatory network that is specific in each gene and cell type in which it is active.[24] For example, it has been shown that HNF1α acted synergistically with octamer transcription factor-1 to enhance the *UGT2B7* promoter activity in HepG2 cells.[25] In contrast, pre B cell homeobox-2 decreased the binding and transcriptional capacity of HNF1α to the *UGT2B17* promoter.[26] Although little is known about regulatory networks for transcription control in the *UGT1A* genes,

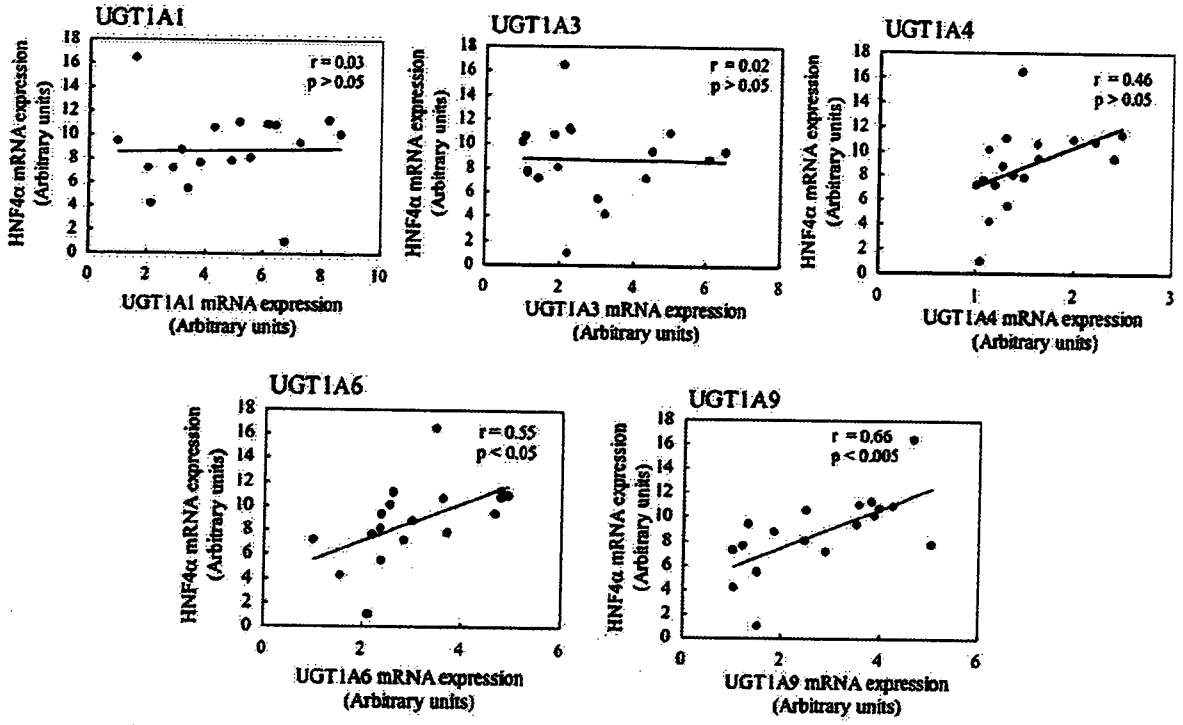

Fig. 4. Correlations between expression levels of UGT1A mRNA (UGT1A1, 1A3, 1A4, 1A6 and 1A9) and HNF4α mRNA in human livers. The correlations between the amounts of UGT1A mRNA and the amount of HNF4α mRNA were analyzed by linear regression analysis. The correlation coefficient (r) and significant value (p) for each correlation is shown.

the results of these studies imply that the relationship between HNF1α and HNF4α expression and UGT1A1, UGT1A3 and UGT1A4 expression might be complicated by interactions with other transcription factors.

Among the hepatic UGT1A isoforms, we observed the greatest interindividual difference in UGT1A1 mRNA expression (**Fig. 1**). It has been reported that expression of the *UGT1A1* gene can be modulated by hormones, drugs and other xenobiotics through interaction with the aryl hydrocarbon receptor and members of the nuclear receptor superfamily, including constitutive androstane receptor, pregnane X receptor and peroxisome proliferator-activated receptor alpha.[10,27,28] Therefore, in addition to the expression levels of transcription factors, the variability of UGT1A1 mRNA level might be explained by drugs, including phenytoin, phenobarbital and dexamethasone, and/or tobacco consumption, which can activate these receptors to bind to the promoter region of the *UGT1A1* gene.[8] Although data on environmental factors are not available for all the liver samples, we could not find any significant relation of drugs used to the variability of UGT1A1 mRNA level in the samples of which the environmental data are available. In addition to environmental factors, genetic polymorphisms in the promoter region have been considered as factors determining the variability of UGT1A1 expression.[8] It has been reported that transcription activity of the *UGT1A1* gene is reduced by the length of the TATA box (*UGT1A1*28*, $(TA)_7TA$) and polymorphism in phenobarbital-responsive enhancer module (*UGT1A1*60*, T3263G).[8,29] Although we did not analyze these variants in the present study, it is possible that the variation in UGT1A1 mRNA level in the liver samples studied can be partly explained by genetic polymorphisms.

Finally, it should be noted that liver tissues used in the present study were macroscopically normal part of tissues surrounding tumor areas, which were removed during the surgery of metastatic tumor in the liver. Thus, the results of the present study should be interpreted with the limitation that they were derived from the liver tissue samples obtained from the normal part of the liver bearing tumor(s) but not from the normal liver.

In conclusion, our results suggest that both HNF1α and HNF4α are the factors involved in the interindividual variability of UGT1A6 and UGT1A9 mRNA expression levels at least in human livers used in this study. Although our results did not rule out the possibility of involvement of HNF1α and HNF4α in the regulation of hepatic UGT1A1, UGT1A3 and UGT1A4 expression, these transcription factors are unlikely to be factors determining of the interindividual variability of UGT1A1, UGT1A3 and UGT1A4 mRNA expression levels in human livers. Further studies aimed at identification of

other transcription factors and their roles in regulatory networks are needed to clarify the causal factors of the interindividual differences in UGT1A1, UGT1A3 and UGT1A4 mRNA expression levels in human livers.

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Short Communication

Hepatocyte Nuclear Factor 1 Alpha is a Factor Responsible for the Interindividual Variation of OATP1B1 mRNA Levels in Adult Japanese Livers

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Purpose. The aim of the present study was to clarify the factors responsible for interindividual variability of organic anion transporting polypeptide (OATP, gene symbol *SLCO*) 1B1 mRNA expression level in the human liver.
Materials and Methods. OATP1B1 mRNA expression levels were determined by real-time PCR in 31 human liver samples. The results were analyzed in relation to a single nucleotide polymorphism (−11187G>A) located in the promoter region and levels of hepatocyte nuclear factor (HNF) 1α mRNA.
Results. There was a 4.9-fold interindividual variability of OATP1B1 mRNA expression level in the livers analyzed, which was not associated with −11187G>A polymorphism. Accordingly, the −11187G>A polymorphism did not alter the *SLCO1B1* gene promoter activity in luciferase assays. On the other hand, OATP1B1 mRNA levels showed a significant correlation with HNF1α mRNA levels (r=0.83, P<0.0001). This correlation was consistent with the results of luciferase assays and chromatin immunoprecipitation assays showing functional interaction between HNF1α and *SLCO1B1* gene promoter.
Conclusions. Our results suggest that HNF1α is an essential regulator of OATP1B1 mRNA expression and thus the level of HNF1α expression is one of the major determinants of interindividual variability in OATP1B1 mRNA expression.

KEY WORDS: hepatocyte nuclear factor 1α; interindividual variability; liver; organic anion transporting polypeptide 1B1; transcriptional regulation.

INTRODUCTION

Human organic anion transporting polypeptide 1B1 (OATP1B1, gene symbol *SLCO1B1*) is specifically expressed in the liver, where it mediates basolateral uptake of various compounds from circulation. Structurally diverse drugs, such as HMG-CoA reductase inhibitors and rifampicin, have been reported to be its substrates, making OATP1B1 one of the major determinants for hepatic disposition of substrate drugs (1). OATP1B1 also contributes to liver physiological functions by transporting endogenous organic anions, such as bile salts, bilirubin conjugates, thyroid hormones, and eicosanoids (1,2). Therefore, gain or loss of transport activity of OATP1B1 in individuals could have a great impact on their pharmacokinetic profiles of substrate drugs and physiological homeostasis.

It is thought that there are two main reasons for the interindividual variability of the level of transporting activity of OATP1B1: (1) existence of genetic amino acid variation at a functionally important position in the polypeptides and (2) increased or reduced level of expression of the *SLCO1B1* gene. With respect to the first reason, several single nucleotide polymorphisms (SNPs) have been characterized. One of them, 521T>C (174V>A), has been shown to impair membrane localization of OATP1B1 in cultured cells, eventually resulting in remarkable loss of the function of the transporter (3). Consistent with this *in vitro* finding, it has been reported that the carriers of this SNP showed significant increase in plasma pravastatin concentrations (4,5). This SNP, together with 388A>G (130N>D), has also been reported to be one of the genetic factors related to unconjugated hyperbilirubinemia (6). On the other hand, with respect to the second reason, some researchers have shown that hepatocellular carcinoma and primary sclerosing cholangitis tend to decrease the level of *SLCO1B1* gene expression (7–10). More recently, Ho *et al.* (11) have examined the expression profiles of *SLCO1B1* and *SLCO1B3* genes in 22 human livers, the results of which suggest the existence of interindividual variability in the level of *SLCO1B1* gene expression. The mechanisms by which *SLCO1B1* gene transcription is regulated likely play some roles in the variability of

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ABBREVIATIONS: HNF, Hepatocyte nuclear factor; MODY, Maturity-onset diabetes of the young; OATP, Organic anion transporting polypeptide; PCR, Polymerase chain reaction; SLCO, Solute carrier organic anion transporter; SNP, Single nucleotide polymorphism.

expression levels. Hepatocyte nuclear factor (HNF) 1α has been demonstrated to be a strong activator of the *SLCO1B1* gene promoter in HepG2 cells (12). The SNP (−11187G>A) has been identified within the promoter region of the *SLCO1B1* gene, and the associations of the SNP with increased pravastatin concentration and blood glucose response to repaglinide have also been reported (4,13). However, *in vivo* contribution of HNF1α to *SLCO1B1* gene transcription has not been clarified and other factors that are involved in *SLCO1B1* gene transcription remain unidentified. Effects of the SNP (−11187G>A) on expression level of the *SLCO1B1* gene has been unclear. Therefore, the factors causing the variability in *SLCO1B1* gene expression remain unknown.

In the present study, we determined the levels of OATP1B1 mRNA expression in liver samples obtained from 31 Japanese patients who had undergone hepatectomy. To identify the causal factors for the interindividual variability, we analyzed the relationships of OATP1B1 mRNA expression level with SNP (−11187G>A) and expression level of HNF1α mRNA.

MATERIALS AND METHODS

Human Liver Tissues

Liver tissues were obtained from the National Cancer Center Hospital East (Kashiwa, Japan) and from the International Medical Center of Japan (Tokyo, Japan). The liver samples were normal parts surrounding tumor areas that had been surgically resected from patients with hepatocarcinoma. The hepatocarcinomas in patients resulted from metastasis from colorectal or gastric cancer. The liver tissues were snap-frozen in liquid nitrogen and stored at −80°C or in liquid nitrogen until used. Liver information is shown in Table I. Non-frozen liver tissues (Liver I and Liver II) were supplied by the National Disease Research Interchange (Philadelphia, PA) through the Human and Animal Bridging Research Organization (Tokyo, Japan). Liver I was from an American-African female patient (34 years old), and Liver II was from a Hispanic female patient (35 years old). These liver tissues had been rejected for liver transplantation. All of the patients were negative for hepatitis B virus and hepatitis C virus. Full permission was obtained from the Ethics Committee of Chiba University, Japan, based on the Helsinki declaration.

Table I. Characteristics of Japanese liver samples

Number	Gender	Age (years)
401	Female	76
501	Female	52
601	Male	64
901	Male	49
1001	Male	67
1101	Male	67
1301	Male	71
1401	Male	64
1801	Male	54
2201	Male	56
2301	Male	70
2401[a]	Female	29
2501	Female	71
2601	Male	75
2701	N/A	N/A
2801	N/A	N/A
2901	Male	62
3001	Male	47
0	N/A	N/A
1	Male	60
3	Male	N/A
19[a]	Male	51
20[a]	Male	56
21	Male	59
26	Male	62
34[a]	Female	44
36	Male	53
38	Female	55
39	Male	64
40[a]	Male	64
42	Male	62

Hepatic metastasis from colorectal or gastric cancer was existed in all patients.
N/A, data not available.
[a] Heterozygote carriers of −11187G>A.

Total RNA isolation, cDNA synthesis, and quantitative real-time polymerase chain reaction (real-time PCR)

Total RNAs were extracted from liver tissues by using an SV Total RNA Isolation System (Promega, Madison, WI). Quality of total RNA was assessed by determination of the ratio of 28S rRNA to 18S rRNA by densitometric analysis. No genomic DNA contamination was confirmed by performing PCR as described previously (14). The method of cDNA synthesis was described elsewhere (14).

Real-time PCR was performed by using Taqman Gene Expression Assays with an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City CA) as described previously (14). The probe and primers were Hs00272374-m1 (*SLCO1B1*) and Hs00167041-m1 (*HNF1α*). Four house-keeping genes, beta-glucronidase, cyclophilin, acidic ribosomal protein and glyceraldehyde-phosphate dehydrogenase, were selected as internal standards based on the results of preliminary tests using a Taqman Human Endogenous Control Plate (Applied Biosystems) and previously reported methods in which the levels of expression of target genes were normalized by the averaged value of multiple internal standard genes (15–17). The data were expressed as arbitrary units with the lowest value set to 1. All assays were performed at least three times in triplicate, and analyses of most samples were performed twice with independent total RNA isolation, reverse transcription and real-time PCR.

Genomic DNA Isolation and DNA Sequencing for Genotyping

Genomic DNA was isolated from liver tissue by using a Charge Switch gDNA Micro Tissue Kit (Invitrogen, Carlsbad, CA). An SNP of the *SLCO1B1* gene (−11187G>A, translation start codon=+1) was analyzed by PCR and DNA

sequencing. The primers used for PCR were 5′-CCCATGAA TGATAAGGGGTAACCATA (sense) and 5′-ACTTGACT TGTGGAGAAAGGAAGAGC (anti-sense). The procedure used for DNA sequencing was described previously (18).

Luciferase Reporter Plasmid Construction and Site-directed Mutagenesis

The 2068-bp 5′-upstream region of the *SLCO1B1* gene was cloned from genomic DNA prepared as described above by PCR with the primers 5′-CCTGAGAACTCTGGGGC TAAAACCT (sense) and 5′-AAGACCATCAAAATCT TCTTCCGA (anti-sense). The obtained genomic DNA fragment was inserted into a pCR-Blunt II TOPO vector (Invitrogen), and the DNA sequence was analyzed. Then the upstream region was transferred to a pGL3-basic vector (Promega). This wild-type reporter construct was named pSLCO1B1/wt. To obtain a reporter plasmid harboring −11187G>A, site-directed mutagenesis was performed by methods described previously (19) with the primers 5′-TGTGCATATGTGTATACAAGTAAAAGTGTGTAT (sense) and 5′-ATACACACTTTTACTTGTATACACATA TGCACA (anti-sense). The underlines indicate the position of −11187G>A. The reporter construct harboring a mutation was named pSLCO1B1/mt. The mammalian expression vector of human HNF1α was kindly provided Dr. Ryuichirou Satoh (Lab. of Food Biochemistry, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo).

Cell Culture, Transient Transfection and Dual Luciferase Assay

HepG2 cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum and penicillin/streptomycin.

Transient transfection was performed as described previously (14) with 200 ng/well of the reporter plasmid, 4 ng/well of phRL-TK (Promega). Either HNF1α expression plasmid (pHNF1α) or pTarget empty plasmid (50 ng/well) was added to the transfection mixture in a cotransfection assay. After 24 h of incubation, the cells were harvested and the luminescence was determined as described previously (20).

Preparation of Nuclear Extracts from Hepatocytes and Gel Mobility Shift Assay (GMSA)

Nuclear extracts were prepared from HepG2 cells and primary hepatocytes as described previously (19). The primary hepatocytes were prepared from the non-frozen liver tissue described above at the Human and Animal Bridging Research Organization, and they were immediately used for nuclear extraction. Protein concentration was determined by using a Bio-Rad Dc Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA).

The methods used for GMSA were described in detail in our previous report (14). Oligonucleotide sequences containing wild-type or mutant nucleotides are 5′-TGTGCATAT GTGTATACAGGTAAAAGTGTGTAT (wild type) and 5′-TGTGCATATGTGTATACAAGTAAAAGTGTGTAT (mutant). Only sense strands are shown.

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation assays were performed by using a ChIP-IT kit (Active Motif, Carlsbad, CA). The method for preparation of sheared chromatin from the human liver (Liver I and Liver II) was based on the report of del Castillo-Olivares *et al.* (21). A piece of human liver (800 mg) was isolated and chopped on ice. Cross-linking of proteins to DNA was done by adding 1% formaldehyde in PBS to the chopped liver for 12 min with gentle rotation. To stop the reaction, an equal volume of 250 mM glycine in PBS was added to the mixture for 5 min with rocking. The tissue debris was collected by centrifugation at 2,500 g for 3 min and washed with PBS twice. The pellet was resuspended with PBS containing phenylmethylsulfonyl fluoride and homogenized by a dounce homogenizer. After centrifugation at 2,500 g for 10 min, the sample was resuspended in the lysis buffer supplied with the ChIP-IT kit. The chromatin was sheared by using an Ultrasonic disrupter UD-201 (TOMY, Tokyo, Japan) at 25% power with six pulses. For immunoprecipitation, 12 μg of the sheared chromatin was mixed with either 3 μg of control goat antibodies (sc-2028, Santa Cruz Biotechnology, CA, USA) or 3 μg of anti-HNF1α IgG (sc-6547x, Santa Cruz Biotechnology). After incubating for 12 h at 4°C with gentle rotation, the following procedure was used as described in a previous report (14). PCR was performed with two sets of primers at 38 cycles. One set of primers was for detection of the region containing the HNF1α binding site in the *SLCO1B1* promoter, 5′-TGGCAACTGGAGTGAACTCTTAAAAC (sense) and 5′-TCCTACAGCAACTGCAACAAGTCCAC (antisense), and the other set of primers was for detection of the intronic region between exons 8 and 9, 5′-CCTTCTACTCCTTATTT CAAGCAG (sense) and 5′-ACTGAGAGTTGGATGA TACGGACT (antisense).

Statistical Analysis

The difference between OATP1B1 mRNA expression levels in carriers of the SNP and non-carriers was analyzed by the Mann–Whitney U test. The correlation of levels of OATP1B1 mRNA with those of HNF1α mRNA was analyzed by linear regression analysis. These statistical tests were performed by using Statcel software (OMS Publishing Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

Real-time PCR was performed to determine the level of variability in OATP1B1 mRNA expression in the human liver. The results showed that there was an approximately 4.9-fold difference in the expression level. To identify the factors causing the interindividual difference in the OATP1B1 mRNA expression level, we analyzed the effects of the SNP (−11187G>A) on OATP1B1 mRNA expression level. The SNP was located in the promoter region of the *SLCO1B1* gene, 814 bp upstream from the transcription start site (11187 is the position with respect to the translation start

codon). The SNP has been reported to be associated with increased pravastatin concentration and blood glucose response to repaglinide (4,13), possibly because the SNP may affect the level of *SLCO1B1* gene expression. However, whether the associations are attributed to the effect of the SNP on expression of OATP1B1 mRNA has been controversial due to partial linkage between −11187G>A and 521T>C, the latter of which has been shown to impair the function of OATP1B1 protein. Thus, we analyzed the effects of the SNP on the expression levels of OATP1B1 mRNA by comparing the mRNA levels in G/G (wild-type) homozygotes and G/A heterozygotes of −11187G>A. As shown in Fig. 1A, there was no significant difference between the two groups. We also examined the effect of the SNP on promoter activity by luciferase reporter assays. Consistent with the result described above, the SNP failed to alter promoter activity of the *SLCO1B1* gene in HepG2 cells (Fig. 1B). In addition, we performed GMSA, the results of which showed that the SNP did not affect the binding profile of nuclear proteins prepared from HepG2 cells or human hepatocytes to the oligonucleotides (data not shown). These results suggest that the SNP (−11187G>A) is not functional and that the apparent associations of the SNP with drug pharmacokinetics or response are mainly a result of the effect of 521T>C. Taken together, our results suggest that the SNP (−11187G>A) is unlikely to be a major determinant of OATP1B1 mRNA expression level at least in Japanese livers.

Although little is known about the mechanisms controlling *SLCO1B1* gene transcription, it has been reported that HNF1α is a strong activator of the *SLCO1B1* promoter (12). We confirmed that HNF1α could strongly enhance *SLCO1B1* promoter activity (18-fold increase) in luciferase assays (data not shown). Then, we further performed chromatin immunoprecipitation assays by using two human liver tissues (Liver I and Liver II) to examine *in vivo* binding of HNF1α to the *SLCO1B1* promoter. The results showed that amounts of the *SLCO1B1* promoter region in DNA fragments precipitated with anti-HNF1α antibodies were larger than those in fragments precipitated with control antibodies (Fig. 2), indicating that HNF1α can bind to the promoter region of the *SLCO1B1* gene *in vivo*. Collectively,

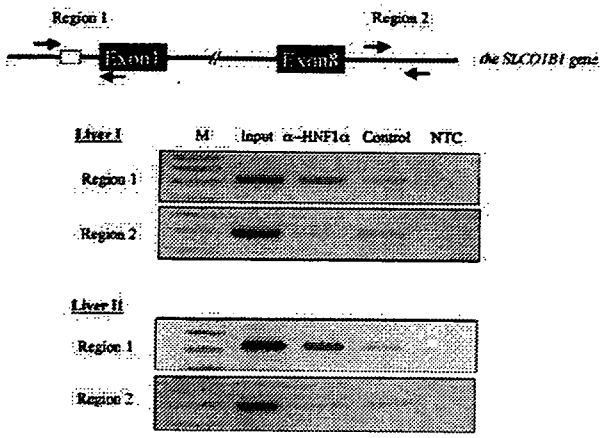

Fig. 2. Interaction between HNF1α and *SLCO1B1* gene promoter. Binding of HNF1α to *SLCO1B1* gene promoter region in the human liver examined by chromatin immunoprecipitation assay. Two human liver tissues (*Liver I* and *Liver II*) were used in this assay. Sheared chromatin was incubated with either control goat antibodies (Control, 3 μg) or anti-HNF1α antibodies (α-HNF1α, 3 μg) for 12 h at 4°C. *Arrows* indicate the positions of primers, and a *white box* indicates an HNF1α binding site. PCR cycles were 38. *M*, DNA marker; *NTC*, non-template control.

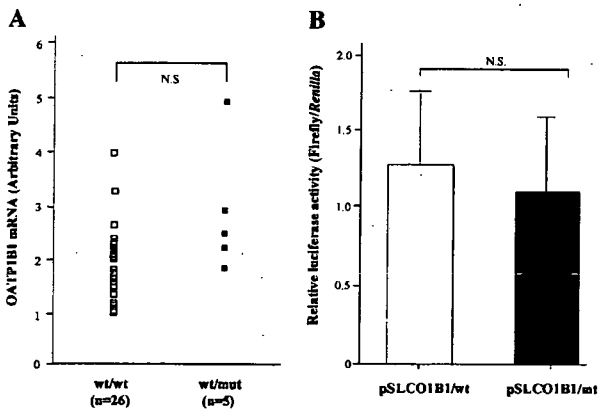

Fig. 1. Effects of the SNP (−11187G>A) on expression level of OATP1B1 mRNA. **A** comparison of OATP1B1 mRNA levels in carriers and non-carriers (*wt/wt*, −11187GG homozygote; *wt/mut*, heterozygote of −11187G and A). Expression levels of OATP1B1 mRNA were determined by real-time PCR. *N.S.*, not significant (Mann–Whitney *U* test). **B** effects of an SNP (−11187G>A) on promoter activity of the *SLCO1B1* gene in HepG2 cells. Results are expressed as means ± S.D. of three experiments, each performed in triplicate. *N.S.*, not significant (Student's *t* test); *pSLCO1B1/wt*, pGL3-Basic vector carrying wild-type 2068 bp of the 5′-upstream region of the *SLCO1B1* gene; *pSLCO1B1/mt*, pGL3-Basic vector carrying 2068 bp with the mutation of −11187G>A.

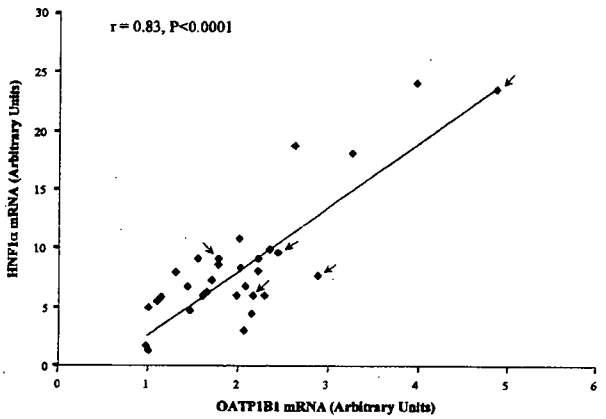

Fig. 3. Correlation between OATP1B1 mRNA expression levels and HNF1α mRNA expression levels in human liver samples. The expression levels of OATP1B1 mRNA and HNF1α mRNA levels in liver samples (n=31) were determined by real-time PCR. Results were normalized by using the mean value of four human endogenous genes (see Materials and Methods). Arbitrary units were calculated with the lowest level of mRNA (# 2801 for OATP1B1 and #1001 for HNF1α) set to unit=1. The values are expressed as means ± S.D. of three separate determinations. Correlation was analyzed by linear regression analysis. *Arrows* indicate the samples carrying the SNP −11187G>A.

HNF1α would play an important role in the regulation of *SLCO1B1* gene transcription in human liver.

Since HNF1α has been shown to be an important factor regulating *SLCO1B1* gene transcription, we examined the association of HNF1α with OATP1B1 mRNA expression in the human liver. As shown in Fig. 3, the levels of HNF1α mRNA varied by 23.8 fold in the samples, and the levels of HNF1α mRNA showed a significant correlation with OATP1B1 mRNA levels (r=0.83, p<0.0001). This correlation was unlikely due to the quality of RNA tested because when compared with UDP-glucuronosyltransferase 1A1 and 1A3 mRNA levels, OATP1B1 mRNA levels were not correlated with either gene (unpublished observation). This finding together with the findings described above suggest that HNF1α is an essential regulator of OATP1B1 mRNA expression and thus the level of HNF1α expression is one of the major determinants of the interindividual variability in OATP1B1 mRNA expression.

It is known that several mutations of the HNF1α gene are responsible for maturity-onset diabetes of the young type 3 (MODY3). MODY3 has been shown to be the most common type of MODY in the United Kingdom and a common type of MODY in German, Danish, Italian, Finnish, North American, and Japanese pedigrees (22,23). It is thought that impaired function of HNF1α caused by a mutation leads to aberrant expression of its target genes. Thus, the expression level of OATP1B1 mRNA may be decreased in patients with MODY3, and pharmacokinetics of drugs transported by OATP1B1 might be altered in such patients. Although there has been no study in which the levels of OATP1B1 expression in the livers of MODY3 patients were examined, it has been reported that *Hnf1α*$^{-/-}$ mice, a model for MODY3, showed lower hepatic uptake of glibenclamide than that in wide-type mice, resulting in higher plasma concentration of the drug (24). Although the murine hepatic glibencramide transporter has not been identified, it has been shown that some hepatic uptake transporters are downregulated in *Hnf1α*$^{-/-}$ mice (25,26). Therefore, it may be worth evaluating the possibility of alteration in the *SLCO1B1* gene expression level or drug disposition in MODY3 patients.

In conclusion, our results show that the expression level of HNF1α but not the SNP (−11187G>A), is a strong candidate for factors causing the variability of OATP1B1 mRNA level observed in human livers. Since it has been reported that HNF1α can interact with various transcription factors, it is likely that HNF1α orchestrates multiple factors on the *SLCO1B1* gene to control its transcription. Such factors are currently unknown, and further study is needed to determine the factors and how they cooperate with HNF1α. The results of such study would reveal the impact of HNF1α on *SLCO1B1* gene transcription, which is one of the cornerstones for further understanding the interindividual variability of OATP1B1 mRNA expression in a steady state as well as in pathological conditions.

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