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Genetic Polymorphisms of *UGT1A* and their Association with Clinical Factors in Healthy Koreans

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Abstract

Glucuronidation by the uridine diphosphateglucuronosyltransferase 1A enzymes (UGT1As) is a major pathway for elimination of particular drugs and endogenous substances, such as bilirubin. We examined the relation of eight single nucleotide polymorphisms (SNPs) and haplotypes of the *UGT1A* gene with their clinical factors. For association analysis, we genotyped the variants by direct sequencing analysis and polymerase chain reaction (PCR) in 218 healthy Koreans. The frequency of *UGT1A1* polymorphisms, -3279T>G, -3156G>A, -53 $(TA)_{6>7}$, 211G>A, and 686C>A, was 0.26, 0.12, 0.08, 0.15, and 0.01, respectively. The frequency of -118 $(T)_{9>10}$ of *UGT1A9* was 0.62, which was significantly higher than that in Caucasians (0.39). Neither the -2152C>T nor the -275T>A polymorphism was observed in Koreans or other Asians in comparison with Caucasians. The -3156G>A and -53 $(TA)_{6>7}$ polymorphisms of *UGT1A* were significantly associated with platelet count and total bilirubin level (p=0.01, p=0.01, respectively). Additionally, total bilirubin level was positively correlated with occurrence of the *UGT1A9*-118 $(T)_{9>10}$ rare variant. Common haplotypes encompassing six *UGT1A* polymorphisms were significantly associated with total bilirubin level (p=0.01). Taken together, we suggest that determination of the *UGT1A1* and *UGT1A9* genotypes is clinically useful for predicting the efficacy and serious toxicities of particular drugs requiring glucuronidation.

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Introduction

Glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGTs), is one of the critical steps in the detoxification and elimination of various endogenous and exogenous compounds. As for the genes encoding UGTs, two subfamilies, *UGT1* and *UGT2*, have been identified in humans (Mackenzie PI *et al*., 1997, Saeki M *et al*., 2006, Sandanaraj E *et al*., 2007). The human *UGT1A* gene complex spans approximately 200 kb and is located on chromosome 2q37 and consists of nine active and four inactive exon 1 segments and common exons 2-5. One of the nine active exon 1s (namely, 1A1 and 1A3-1A10) can be used in conjunction with the common exons (Saeki M *et al*., 2006; Riedy M *et al*., 2000; Kiang TK *et al*., 2005; Sandanaraj E *et al*., 2007). The N-terminal domain of the UGT1A proteins encoded by the first exon is engaged in substrate-binding specificity, and the C-terminal domain encoded by the common exons 2-5 plays a role in binding to UDP-glucuronic acid (Sandanaraj E *et al*., 2007) (Fig. 1).

Several studies have displayed an association of toxicities of particular drugs with the presence of functional polymorphisms in *UGT1A* isoforms, which lead to impairment of the glucuronidation of SN-38 (Sandanaraj E *et al*., 2007; Jada SR *et al*., 2007, Minami *et al*., 2007). In addition, recent studies have suggested possible contributions of *UGT1A7, 1A9,* and *1A10* to the formation of SN-38G (Kiang TK *et al*., 2005; Minami *et al*., 2007). The presence of seven TA tandem repeats [g.-53$(T/A)_{7/7}$; UGT1A1*28] instead of six [g.-53$(T/A)_{6/6}$; UGT1A1*1] in the promoter of *UGT1A1* is associated with not only low rates of SN-38 glucuronidation but also the occurrence of irinotecan-induced severe toxicities.

Additional functional polymorphisms have been discovered in the promoter (UGT1A1*60; g.-3279T>G, and g.-3156G>A) and coding regions (c.211G>A (Gly71Arg), UGT1A1*6; c.1456T>G (Tyr486Asp), UGT1A1*7; c.686C>A (Pro229Gln), UGT1A1*27) of the *UGT1A1* gene (Sandanaraj E *et al*., 2007). Several functional variations and haplotypes of the *UGT1A9* gene are associated with altered gene expression or catalytic activity (Sandanaraj E *et al*., 2007; Han JY *et al*., 2006).

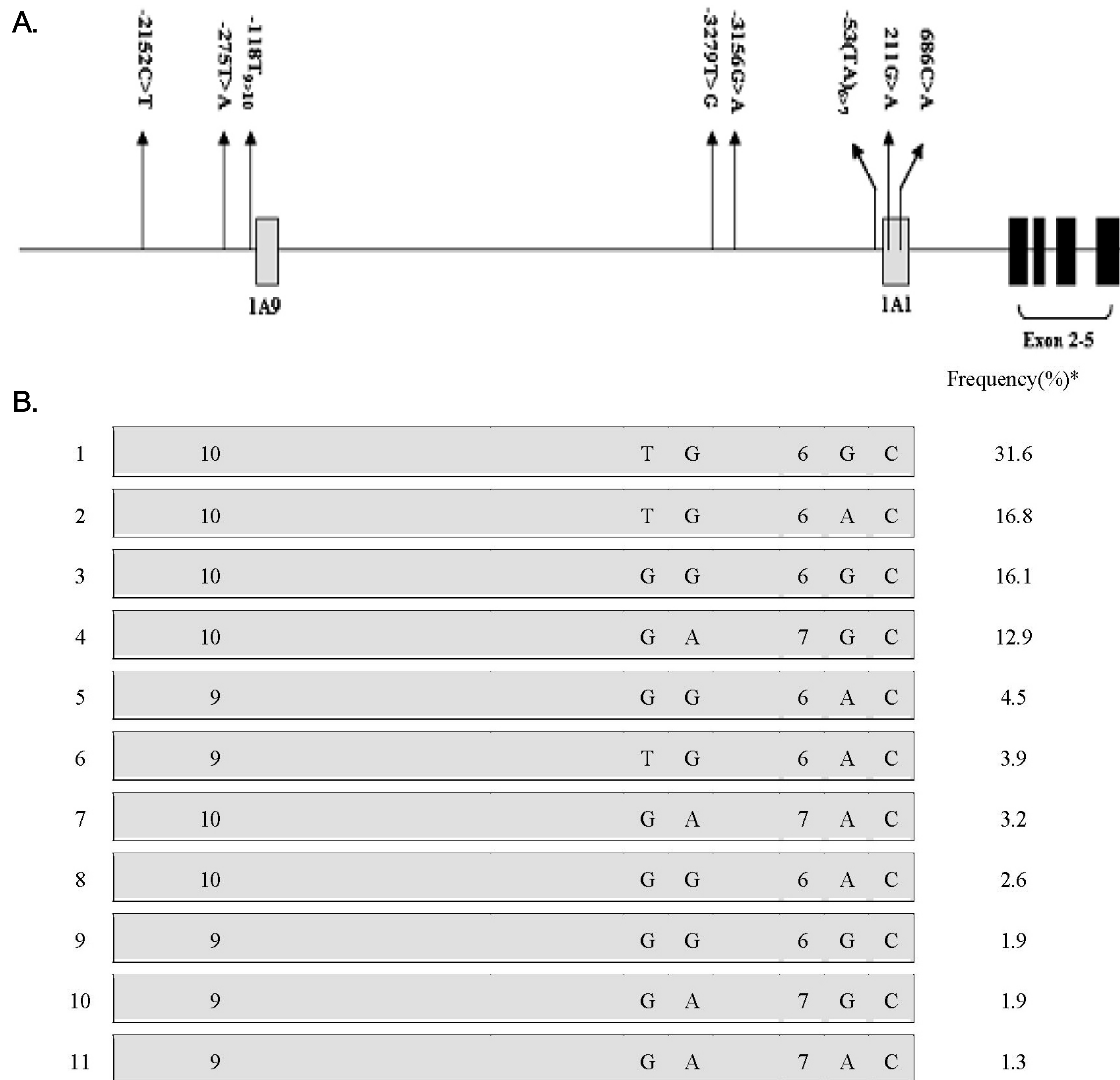


Fig. 1. The genomic structure and polymorphism locations of *UGT1A*. A. The eight SNPs used in this analysis are indicated by arrows. Common exons 2-5 are depicted by black boxes. B. The distribution of haplotypes in *UGT1A1* (-3279T>G, -3156G>A, -53$(TA)_{6>7}$, 211G>A, 686C>A) and *UGT1A9* (-118$(T)_{9>10}$)
*more than 1% of frequency

Recently, Yamanaka *et al*. found that a one-base insertion of thymidine in a promoter region of *UGT1A9* (-118$(T)_{9>10}$, UGT1A9*22) has an influence on variable expression of the UGT1A9 enzyme.

There are significant racial differences in *UGT1A1* and *UGT1A9* polymorphisms among Asians, Caucasians, and Africans. It has been reported that the frequency of the UGT1A1*28 $(TA)_7$TAA allele ranges from 15% in Asians and American Indians to 45% in sub-Saharan Africans and 26% to 38% in Caucasians, Hispanics, and African-Americans (Marsh and McLeod, 2004). Meanwhile, it has been known that the frequencies of -2152C>T, -275T>A, and -118$(T)_{9>10}$ in *UGT1A9* are 3%, 4%, and 39% in Caucasians, respectively (Innocenti *et al*., 2005; Villeneuve *et al*., 2003). Higher frequencies of the -118$(T)_{9>10}$ allele were observed in Asians compared with Caucasians (Innocenti *et al*., 2002).

The goal of the present study is firstly to screen the single nucleotide polymorphisms (SNPs) in the promoter and coding regions of *UGT1A1* and *UGT1A9* in healthy Koreans, and secondly, to analyze an association of *UGT1A1* and *UGT1A9* variants with their clinical and laboratory factors.

Materials and Methods

Subjects

Our research was activated after the Institutional Review Board (IRB) had approved the study protocol. After written consent forms were gathered from 218 healthy Koreans visiting to undergo medical examination at Kangnam St. Mary's hospital between April and August 2005, blood was collected from the peripheral vein. We established a database of clinical information of the study subjects undergoing physical checkups including gender, age, smoking status, biochemical test (albumin, aspartic acid transaminase (AST), alanine transaminase (ALT), total bilirubin, and creatinine), and hematological test (leukocytes, red blood cells, hemoglobin, hematocrit, and platelet). Using an isolation kit (G-DEX™, Korea) containing a spin column, genomic DNA was extracted from 300 uL peripheral blood drawn from 218 individuals. The purity of extracted DNA was measured by a NanoDrop (ND, USA) and then stored at -70℃ until the next experiment.

Laboratory analysis

DNA sequencing of *UGT1A1* and *UGT1A9* and genotyping assay

For the polymerase chain reaction (PCR), 100 ng of genomic DNA, 10 uM of *UGT1A1* primers (3279T>G, -3156G>A, -53 $(TA)_{6>7}$, 211G>A and 686C>A) and *UGT1A9* primers ($118T_{9>10,}$ -275T>A and -2152C>T), deoxyribonucleoside triphosphate (Genemed, Korea), 2.5 uM Ace-Taq polymerase (Genemed, Korea), and 10X PCR buffer were mixed and adjusted to 25 uL and amplified separately using a thermal cycler (Perkin- Elmer, Boston, Massachusetts, USA). Electrophoresis (100 V, 20 minutes) was carried out on 1.5% agarose gel using gel-loading buffer, and DNA amplification was confirmed on an image analysis system. The sequence of primers prepared for PCR and their reaction conditions are precisely described in Appendix A. The amplified PCR products were purified by spin column (MEGA- spin™, Korea), and then we measured the concentration using the NanoDrop (ND, USA). The sequence was analyzed in 218 subjects by the BigDye Terminator V31 Cycle Sequencing kit (Applied Biosystems, Foster, California, USA) and the ABI 3730XL Sequencer (Applied Biosystem, USA). The -53 $(TA)_{6>7}$ (TATA indel) band was confirmed by electrophoresis immediately after PCR, and then without purification, it was directly injected into a DHPLC instrument (denaturing high performance liquid chromatography, WAVE 3500HT analysis). Especially, in the case of -$118T_{9>10}$ among three genetic variants of *UGT1A9*, we re-analyzed and confirmed its DNA sequence using gene scan, considering the possible misinterpretation due to the slippage phenomenon occurring in the first analysis of direct sequencing.

Statistical analysis

All data were analyzed with the Statistical Analysis Systems (SAS) software version 8.01 (SAS Institute, Cary, NC, USA). The clinical characteristics of continuous variables were expressed as mean ± S.D. The inter-ethnic differences of genotype and allele frequencies for *UGT1A1* and *UGT1A9* polymorphisms were calculated using Fisher's exact test. Differences in quantitative variables were calculated by Student's unpaired *t*-test. The bilirubin levels and platelet counts for the different genotypes were compared using ANOVA. Allele frequencies were estimated by the gene-counting method, and an exact test was performed to identify departures from Hardy-Weinberg equilibrium (HWE). Linkage disequilibrium (LD) between pairs of SNPs was assessed using Lewontin's standardized disequilibrium coefficient $|D'|$ and the squared correlation coefficient (r^2). An expectation-maximization (EM) algorithm was used to estimate the haplotype frequencies. A dominant logistic regression analysis was used for the evaluation of the independent effect of the investigated genetic variants, adjusted for the presence of factors.

Appendix. A. The primer sequences designed for six polymorphic variants of *UGT1A1* and *UGT1A9*.

Gene	polymorphism	Sequence of primer (5' to 3')	Annealing Temperature
UGT1A1	-53$(TA)_{6>7}$	Forward: GTCACGTGACACAGTCAAAC Reverse: TTTGCTCCTGCCAGAGGTT	55°C
	-3279(T>G) -3156(G>A)	Forward: CTCGGGATAAACATGGGATG Reverse: CACCACCACTTCTGGAACCT	61°C
	211(G>A) 686(C>A)	Forward: ATGCTGGGAAGATACTGTTG Reverse : TTTGGTGAAGGCAGTTGATT	58°C
UGT1A9	-118$(T)_{9>10}$	Forward: CATAAGCTACTGTTGTCTGGA Reverse: GTTTCTTTGTGCAAGCCATCAGAGAAC	60°C
	-275(T>A)	Forward: TGGACAGAGAGTATTTGGTTGC Reverse: TGTGCTGCAATGTTAAGTTTAG	60°C
	-2152(C>T)	Forward: TGCTAGTACATTTGACCCTTG Reverse: TGGTGAAACCCTATCTCTACT	60°C

P-values less than 0.05 were considered as indicative of statistical significance.

Table 1. Baseline characteristics of healthy Koreans

	Mean	Range
Total (N)	218	
Sex (M : F)	140 : 78	
Age	45.9	22-72
Height (cm)	166.8	150-187
Weight (kg)	65.6	41-99
BSA	1.7	1.3-2.2
Hematology		
WBC (10^9/L)	5.7	2.6 -12.9
RBC (10^{12}/L)	6.9	3.7-17.7
Hemoglobin (g/dL)	14.5	9.9-17.3
Hematocrit (%)	42.5	32.7-56.5
Platelet (10^9/L)	239	101-401
Biochemistry		
Albumin(g/dl)	4.4	3.7-5.0
AST (U/L)	26.1	13-118
ALT (U/L)	26.1	8-106
Total bilirubin (mg/dl)	0.57	0.19-1.97
Creatinine (mg/dl)	1.0	0.6-1.5

WBC, white blood cell; RBC, red blood cell; AST, aspartic acid transaminase; ALT, alanine transaminase.

Results

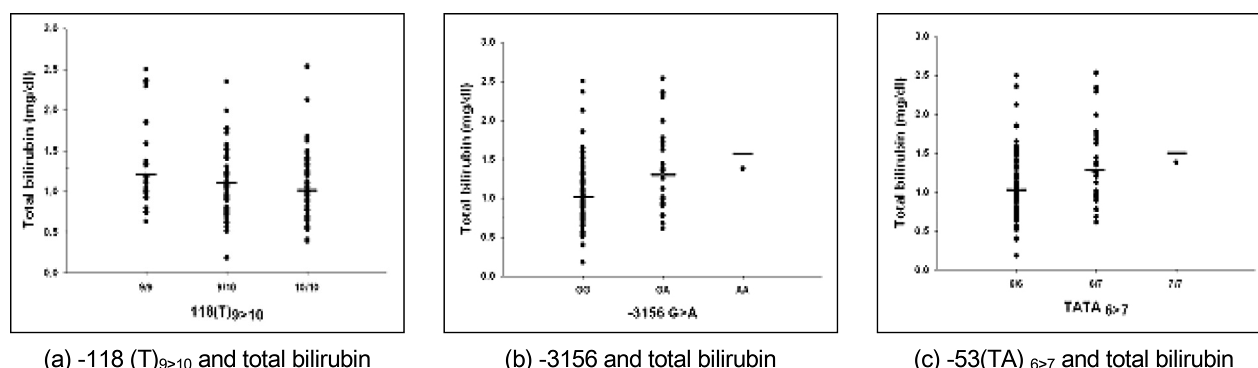
Baseline characteristics of subjects and the distribution of genetic polymorphisms in *UGT1A1* and *UGT1A9*

The male-to-female ratio of 218 healthy individuals was 140:78, and their mean age was 45.9 years (range 22-72 years); the results of the hematological and biochemical tests are summarized in Table 1. The distribution of *UGT1A1* polymorphisms -3279T>G, -3156G>A, -53 $(TA)_{6>7}$, 211G>A, and 686C>A was 0.26, 0.12, 0.08, 0.15, and 0.01, respectively. The frequency of -118 $(T)_{9>10}$ of *UGT1A9* (0.62) in healthy Koreans was significantly higher than in Asian and Caucasian subjects (0.53 and 0.39, respectively). Neither the -2152C>T nor the -275T>A polymorphism was observed in Koreans or Asians-only in Caucasians. The genotype distributions for all variants were in HWE. However, there was no significant deviation from HWE in the genotype frequencies of the -3279T>G polymorphism (p=0.019). The measure of LD was calculated for all combinations of SNPs using the Lewontin's |D'| and r^2 coefficients (data not shown). Strong LD was found in -3156G>A with -53$(TA)_{6>7}$ and 211G>A variants.

Table 2. The association of UGT1A(UGT1A1,UGT1A9) with clinical factors

	white blood cell (10^9/L)		red blood cell (10^{12}/L)		Hemoglobin (g/dL)		Hematocrit (%)		Platelet (10^9/L)		Creatinine (mg/dl)		Total bililubin (mg/dl)		Albumin (g/dl)		AST (U/L)		ALT (U/L)	
	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue	M±SD	Pvalue
-3279T>G																				
TT	5.7±1.4		4.7±0.5		14.5±1.5		42.6±4.2		247.3±44.8		1.0±0.2		1.0±0.3		4.5±0.2	0.49	28.1±12.2	0.49	28.8±17.7	0.22
TG	5.9±1.6		4.7±0.6		14.5±1.6		42.7±4.2		243.8±51.1		1.1±0.2		1.2±0.5		4.4±0.2		26.1±12.9		24.9±14.0	
GG	5.6±1.4	0.79	4.4±0.5	0.4	13.7±1.6	0.53	40.2±4.4	0.43	218.4±101.9	0.44	1.0±0.2	0.16	1.0±0.3	0.15	4.5±0.2		23.2±6.9		20.6±7.8	
-3156G>A																				
GG	5.8±1.4		4.7±0.5		14.5±1.5		42.5±4.0		246.1±49.7		1.0±0.2		1.0±0.4		4.5±0.2		27.8±13.5		27.9±17.3	
GA	5.8±1.8		4.8±0.7		14.6±1.7		43.0±4.8		244.4±44.6		1.0±0.2		1.3±0.6		4.5±0.2		24.2±4.7		22.4±8.6	
AA	4.7±@	0.74	4.5±@	0.47	12.7±@	0.45	37.0±@	0.35	101±@	0.01	1.0±@	0.72	101±@	0.002	4.3±@	0.63	28.0±@	0.38	27.0±@	0.24
-53(TA) 6>7																				
6/6	5.8±1.4		4.7±0.5		14.5±1.5		42.6±4.0		245.7±49.1		1.0±0.2		1.1±0.4		4.5±0.2		27.8±13.6		28.1±17.4	
6/7	5.8±1.7		4.8±0.7		14.5±1.8		42.7±4.9		246.1±47.5		1.0±0.2		1.3±0.5		4.5±0.2		24.2±4.9		22.4±8.4	
7/7	4.7±@	0.75	4.5±@	0.72	12.7±@	0.5	37.0±@	0.4	101.0±@	0.01	1.0±@	0.76	1.4±@	0.004	4.3±@	0.57	28±@	0.34	27.0±@	0.21
211G>A																				
GG	5.8±1.4		4.8±0.5		14.7±1.4		43.1±4.0		245.6±50.6		1.0±0.2		1.1±0.4		4.5±0.2		28.2±14.2		28.1±16.8	
GA	5.8±1.7		4.6±0.6		14.1±1.7		41.7±4.5		244.9±49.7		0.9±0.2		1.1±0.5		4.4±0.2		24.9±6.9		24.7±14.7	
AA	5.1±0.9	0.63	4.4±0.5	0.21	13.8±1.7	0.08	41.0±5.5	0.14	225.5±30.7	0.73	0.9±0.1	0.12	1.1±0.1	0.42	4.7±0.1	0.13	24.7±6.4	0.28	19.8±6.8	0.33
686C>A																				
CC	5.8±1.5		7.0±33.5		14.5±1.5		42.5±4.1		244.1±49.6		1.0±0.2		1.1±0.4		4.5±0.2		27.1±12.4		26.9±16.2	
CA	5.0±1.1		4.7±0.7		13.8±2.1		40.8±5.2		280.7±58.5		0.9±0.2		0.9±0.09		4.6±0.1		24.0±8.9		22.7±7.2	
AA	@	0.33	@	0.32	@	0.56	@	0.43	@	0.12	@	0.47	@	0.81	@	0.4	@	0.76	@	0.33
-118(T)9>10																				
9/9	5.7±1.2		4.8±0.7		14.4±1.5		42.4±3.9		230.7±50.8		1.0±0.2		1.3±0.6		4.4±0.3		25.2±6.9		24.5±10.2	
9/10	5.8±1.4		4.6±0.4		14.4±1.5		42.2±4.1		248.6±50.2		1.0±0.2		1.0±0.4		4.5±0.2		27.2±14.1		27.0±18.2	
10/10	5.8±1.7	0.91	4.8±0.6	0.1	14.6±1.6	0.67	43.1±4.4	0.45	246.3±49.0	0.3	1.0±0.2	0.92	1.1±0.4	0.01	4.5±0.2	0.19	27.7±12.0	0.69	27.4±15.6	0.74

P value was obtained from ANOVA test; M, Mean; SD, standard deviation; @, indicates technical difficulty in obtaining statistical description due to small number of subjects



(a) -118 $(T)_{9>10}$ and total bilirubin (b) -3156 and total bilirubin (c) -53(TA) $_{6>7}$ and total bilirubin

Fig. 2. The association between the genotypes of three polymorphisms of UGT1A and total bilirubin level.

The association of individual SNPs and haplotypes with clinical factors

The genotype distribution patterns for homozygotes and heterozygotes for the -3156G>A and -56 $(TA)_{6>7}$ polymorphisms were not significantly different. However, their differences have statistically significant associations with platelet count (p=0.01 for both) and total bilirubin level (p=0.002 and 0.004, respectively) (Table 2). We also found significant associations between total bilirubin levels and a particular polymorphism of *UGT1A9* (-118$(T)_{9>10}$), but not with -3279T>G, -211G>A, or 686C> A polymorphic sites (Table 2, p=0.01).

In a study of association with clinical factors, a higher total bilirubin level was observed in heterozygous carriers of -3156G>A and -53 $(TA)_{6>7}$ compared with homozygotes. Inversely, the total bilirubin level in heterozygous carriers of -118 $(T)_{9>10}$ was lower than in homozygotes (Fig. 2A, B, and C). In addition, it was discovered that platelet counts in heterozygous carriers were lower than in homozygotes of -3156G>A and -53 $(TA)_{6>7.}$

There were possible haplotypes derived from all polymorphic sites in healthy Koreans. Common haplotypes 1 (10-T-G-6-G-C), 2 (10-T-G-6-A-C), and 3 (10-G-G-6-G-C) were distributed more frequently in subjects (31.6%, 16.8%, and 16.1%, respectively) (Fig. 1). In contrast, the distribution of haplotype 10 (9-G-A-7-G-C) and haplotype 11 (9-G-A-7-A-C) was lower in subjects. Common haplotypes encompassing six *UGT1A9* and *UGT1A1* polymorphisms were associated with bilirubin levels (p=0.01).

Discussion

Recent studies have shown differences in disposition of irinotecan and its metabolites among several ethnic groups. These inter-ethnic variations are attributed to a genetic basis resulting from occurrence of functionally relevant SNPs in genes encoding the various drug-metabolizing enzymes, such as UGT1A, and drug transporters, such as the ABC (ATP-binding cassette) protein, engaged in the chemical pathway of irinotecan (Jada SR *et al*., 2007).

In the present study, the polygenic influence of functional *UGT1A1* -3279T>G, -3156G>A, -53 $(TA)_{6>7}$, 211G>A, and 686C>A polymorphisms and *UGT1A9* -118$T_{9>10,}$ -275T>A, and -2152C>T polymorphisms $_{[STK3]}$was evaluated in healthy Koreans to compare with other ethnic groups to further understand the mechanistic basis of inter-ethnic variation of irinotecan-induced toxicities. In our study, it could be postulated that inter-ethnic differences exist in the metabolic profiles and pharmacodynamic effects of irinotecan, although we did not directly investigate racial differences in clinical toxicities induced by irinotecan treatment.

Large differences in the distributions of the *UGT1A1* polymorphisms between Caucasian and Japanese populations have been reported; the frequency of *UGT1A1*28* in Caucasians is higher than that in Japanese. On the contrary, *UGT1A1*6* and *UGT1A1*27*, the variant sequences in exon 1, have been identified only in the Japanese population. Although the clinical significance of irinotecan chemotherapy in cancer patients having these genotypes in exon 1 remains uncertain, they might render Japanese patients to be more sensitive to irinotecan than Caucasians. These findings suggest the importance of racial differences of *UGT1A1* genotypes in irinotecan-induced toxicities (Ando Y *et al*., 2000).

In the present study, -3156G>A and -53 $(TA)_{6>7}$ in *UGT1A1* were significantly related to platelet count (p=0.01 and 0.01, respectively) and total bilirubin level (p=0.002 and 0.004, respectively). We also observed that the total bilirubin level was positively correlated with occurrence of the rare *UGT1A9* -118 $(T)_{9>10}$ variant (p=0.01). These data were substantiated in haplotype

analysis. Common haplotypes were shown to be associated with increased total bilirubin level. We should strive to understand the clinical importance of *UGT1A1* -3156G>A, -53 $(TA)_{6>7}$, and *UGT1A9* -118 $(T)_{9>10}$ with regard to irinotecan-induced toxicities. Thus, this retrospective research warrants a prospective study to corroborate the usefulness of genetic screening for *UGT1A1* and *UTGT1A9* polymorphisms prior to administration of irinotecan.

Beyond our expectation, it was found that platelets count is correlated with these genetic variants with statistical significance. Nowell *et al*. reported the existence of a UDP-glucuronic acid-bilirubin conjugation system in ADP-activated human platelets and identified the expression of the *UGT* enzyme in platelet fractions. Our observation is consistent with their result that $platelets_{[STK4]}$ are involved in multiple steps of heme and bilirubin metabolism (Nowell *et al*., 1998).

Individualization of drug dosage is critical for cancer chemotherapy to reduce unnecessary toxicity and to improve its therapeutic efficacy, because its therapeutic index is often very narrow. Oncologists traditionally have practiced determining the dosage of chemotherapeutic drugs based on the patient's physiological factors (*e.g*., age, body surface area), pathological conditions (*e.g.*, performance status, organ function), and clinical history (*e.g*., previous treatment). If there is a recognized difference in drug disposition and sensitivity caused by the polymorphic drug-metabolizing enzyme, the optimal dosage required for response with the least toxicity could be different in patients with these different genotypes.

We should mention that several biases can modify the distributions of the *UGT1A1* and *UGT1A9* polymorphisms in this study. Nevertheless, it is suggested that the determination of the *UGT1A1* and *UGT1A9* genotypes for irinotecan treatment is useful in oncology.

In summary, comprehensive analysis of *UGT1A1* and *UGT1A9* genotypes showed that these genes are strongly linked to each other, and that the interactions among functional polymorphisms are related to the alteration in the activity of these enzymes. Although it is still hypothetical, we suggest that the *UGT1A1* -3156G>A, -53 $(TA)_{6>7}$, and *UGT1A9* -118 $(T)_{9>10}$ genotypes are important for predicting severe toxicities and treatment outcomes of irinotecan-based chemotherapy.

Acknowledgments

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